Evaluating the permeability and lethality of reverse micelles on *S. cerevisiae* and HeLa cells

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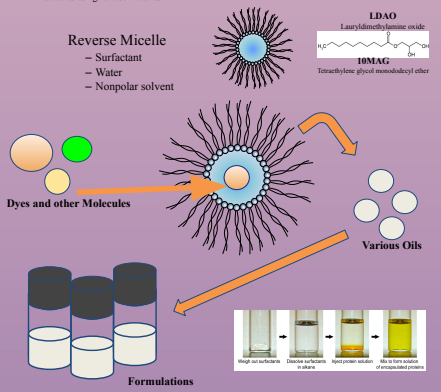
Abstract

Over the last decade, the pharmaceutical industry has seen increasing potential usage of protein therapeutics for drug delivery. However, the delivery of these protein-based drugs and their ability to survive limits their capability. Proteins have short half-lives in organisms and, due to their high molecular weight, they are prone to enzymatic degradation, which decreases their effectiveness. Recent advances in protein engineering and material science have made the targeted delivery of enzyme therapeutics using nanocarriers a new model of treatment. Emulsification techniques are used to encapsulate molecules in nano-sized particles, which are manufactured when two immiscible liquids are mixed to form a single phase by means of an emulsifying agent. By encapsulating molecules, they have enhanced stability, high absorption rates, spontaneous formation, and high solubilization capacity. Using these nanocarrier techniques and various formulations of surfactant and co-surfactants, we tested permeability of these reverse micelles on S. cerevisiae. These reverse micelles containing fluorescent molecules were created using various ratios of surfactant, organic, and water. These formulations were then evaluated for their ability to permeabilize yeast while at the same time evaluating toxicity. Furthermore, we used HeLa cells to test whether these same compounds have any cytotoxic effects against mammalian cells. From our study, we were able evaluate the potential for using reverse micelles as a method for protein-based drug delivery. Results indicated several compounds that were promising for future investigations into this topic.[1][2][3][4]

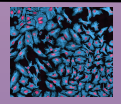
Reverse Micelles

One way that drugs can be encapsulated is through the use of reverse micelles. Reverse micelles are water-in-oil nano-emulsions that use surfactant molecules to disperse aqueous solutes in non-aqueous solvents. Our 10MAG/LDAO mixture is proven to encapsulate a wide range of macromolecules while fully sustaining their structure. [2] Because of this property we are exploring the potential of this mixture as a drug delivery formulation particularly for challenging drug delivery problems including crossing the blood brain barrier and delivery of protein-based therapeutics. Formulations were created using various ratios of surfactants and water in different oils. The formulations included reverse micelles made from surfactants and encapsulated specific dyes. The oils consisted of captex, capmul, lauroglycol, labrafac PG, transcutol HP, capryol 90, plurol oleique, transductol HP, transmutol HP, and isooctane. Choosing to put dyes and other molecules like PI, GFP (green fluorescent protein), and dextran allowed for the observation of different cargo to be delivered into the cells. Propidium iodide (PI) is the initial molecule used for these experiments because of its ability to bind to DNA only when the membrane is permeabilized.[5] Through these many variations, we can determine the boundaries and potentials of delivering molecules using reverse micelles. [7]



HeLa Cells

HeLa cells are an immortal mammalian cell line derived from the cervical cancer cells of Henrietta Lacks, taken from her body in 1951. They are the oldest and most commonly used cell line because of their durability and ability to reproduce rapidly. HeLa cells are inexpensive and easy to culture, making them excellent model organisms for experiments such as these toxicity assays. HeLa cells are very important in scientific research, as they have supported breakthroughs in countless fields since they were first isolated. [10]



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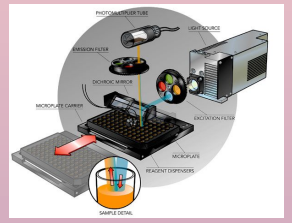
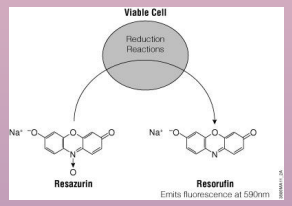
HeLa Cell Permeability



- **Microplate reader** is used to detect chemical, biological or physical reactions by measuring emitted light.[8]



CellTiter-Blue is used to estimate the number of viable cells on given multiwell plates. If the cells are viable they will convert resazurin to resorufin through metabolic reduction reactions.[6]

Examining Lethality of Solvents to HeLa Cells

Solvents	Benefits	Lethality
Isooctane	Low Viscosity, Amenable to Self-Nanoemulsion	Lethal
Captex® 355	Enhances Bioavailablity, Suitable for Emulsions	Not Lethal
Capmul®	Biocompatible, Suitable for Emulsions	Lethal
Capryol® 90	Biocompatible, Suitable for Emulsions	Lethal
Lauroglycol® 90	Enhances Bioavailability, Suitable for Emulsions	Not Lethal
Transcutol® HP	Low Viscosity, Amenable to Self-Nanoemulsion	Lethal
Labrafac® PG	Oily, Good Solubilizer, Suitable for Emulsions	Not Lethal
Plurol Oleique® CC 497	Biocompatible, Enhances Bioavailability, Suitable for Emulsions	Not Lethal

Cytotoxicity Analysis using Hela Cells

CellTiter-Blue Cell Viability Assay of Hela Cells [9] was used to analyze toxicity. After exposure to reverse micelles formulations, HeLa cells were treated with cell-titer blue which has a dye indicator called resazurin to measure cell viability. Viable cells will reduce resazurin to resorufin. Resazurin is a dark blue color and has little intrinsic fluorescence while resorufin is a pink and highly fluorescent. Pink wells indicate viable cells which have successfully reduced resazurin to resorufin.

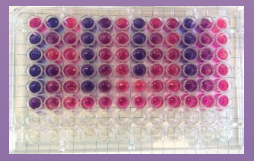
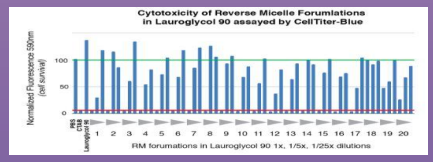


Figure 1: Toxicity for samples of Lauroglycol with PI, including positive and negative controls. Fluorescent signals were normalized to PBS treated cells (living) and CTAB treated cells (cytotoxic). Varying levels of lethality were shown in the different formulations, with a general trend of lower lethality in higher dilutions.



Effects of Reverse Micelles on Cytotoxicity

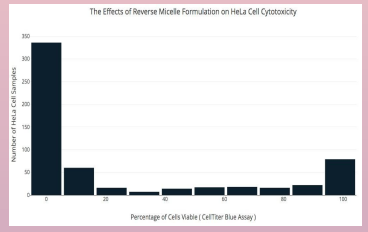


Figure 2: Histogram of HeLa cell viability due to reverse micelle formulations. Results showed a large number of samples with 0% viability, and a moderate number of samples with 50-100% viability when measured with CellTiter-Blue Cell Viability Assay. Formulations that showed 50-100% viability are promising candidates for further experimentation.

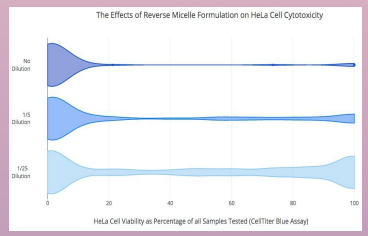
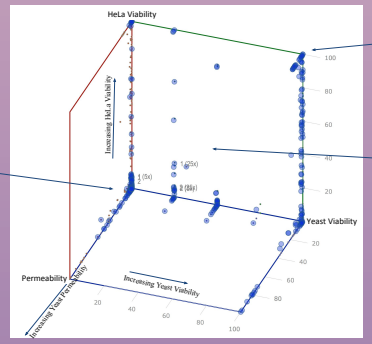


Figure 3: Violin plots of HeLa cell viability due to reverse micelle formulations. Each plot shows the proportion of viable cells in formulations with no dilution, 1:5 dilution, and 1:25 dilution. Viability was minimal in undiluted formulations, but about 50% of samples showed promising viability in formulations with a 1:25 dilution. Results show that a higher proportion of reverse micelles decreases HeLa cell viability in samples.

Compiled Results



Not lethal to Yeast and HeLa *ex. Captex®355 and Lauroglycol® 90*

Lethal to HeLa and yeast cells. *ex.Capmul®*

Ideal: High permeability, Viable for HeLa and Yeast cells

Figure 4: Compiled results of yeast permeability, HeLa lethality, and yeast lethality data. Promising candidate formulations for future studies have high permeability and low lethality to HeLa and yeast cells. Some of these samples include Captex®355 and Lauroglycol® 90.

Conclusions

- Based on our preliminary data, we have successfully been able to distribute dye molecules using reverse micelles into *Saccharomyces cerevisiae* without killing them and have HeLa cells that survived the toxicity of certain formulations
- We have been able to identify several formulations that show promise for future experimentation, including Captex®355 and Lauroglycol® 90.
- While some solvents were not lethal when treating HeLa cells alone, such as captex, lauroglycol, labrafac, and plurol oleique, they were lethal when made into reverse micelle formulations. Further experimentation can be conducted to make new, more effective formulations with these promising solvents.

Future Experiments

- Captex® 355, Lauroglycol® 90, Labrafac® PG, and Plurol Oleique® CC 497 were not lethal to HeLa cells, so should be further studied by creating new formulations with various preparation techniques to see if they become more effective in their permeability and lethality to HeLa cells.
- Further studies can be performed to confirm the effects of Captex®355 and Lauroglycol® 90 on HeLa cell lethality, yeast permeability, and yeast lethality. These studies will provide support for the conclusions made previously, as well as allowing possibility for the creation of similar formulations that may prove more effective.
- Through encapsulating dextran in reverse micelles instead of PI, the maximum size for molecules that will allow for proper cell delivery can be determined; since dextran can range from various molecular sizes.
- When using proteins similar to GFP for encapsulation, the research will be able to focus more towards the primary goal of being able to properly deliver proteins into the cells.
- Analyze the lethality and permeability of Labrafac® PG and Plurol Oleique® CC 497 on yeast cells and compare to HeLa cells
- Most importantly, we hope that the information acquired in this study will help advance a more efficient method for protein-based drug delivery.