Targeted Delivery of Doxorubicin using Folate Bound Lipid Micellar Nanoparticles (LMNs)

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Abstract:

This study observed the relationship between the addition of folate onto Doxorubicin (Dox) loaded lipid micelle nanoparticles (LMN) and the death of MCF-7 breast cancer cells. The study used MCF-7 cells because they over-express folate receptors, which allow folate to act as a targeting molecule when attached to the outside of the micelle. The MCF-7 cells were cultured on a three dimensional (3D) scaffold to form tumoroids and then treated with Dox loaded LMNs, functionalized with increasing amounts of folate. Cellular viability was then analyzed using Presto Blue viability assay. The results showed a correlation between increased amounts of folate attached to the micelles and more efficient killing of MCF-7 cells. More thorough trials will need to be done to determine if this is a viable tumor targeting strategy.

Methods:

- LMNs were synthesized using PEG 2000PE, DC-Cholesterol and DOPE as described in Howell et. al[1] with a significant modification. Folate was attached to outside of the nanoparticles using EDC/NHS coupling chemistry.
- MCF-7 breast cancer cells were used in this study because they are known to over-express folate receptor.
- The folate was bound to the nanoparticles at two concentrations, 30µg and 300µg.
- We found that a polymeric nanofibrous scaffold platform established in our laboratory allows growth of three-dimensional (3D) tumoroids, which resemble *in vivo* tumors. Tumoroids exhibit better drug resistance compared to two-dimensional (2D) cultures that lack ability to mimic the environment of the tumor microenvironment.
- The scaffolds were cut to fit 90 well plates and then cultured with 8000 cells/well and allowed to grow for five days until they formed tumoroids.
- The cells cultures were then treated with various concentrations of each folate LMN/doxorubicin sample.
- After an incubation period of 24 hours the cells were analyzed using Presto Blue cell viability assay.

Objectives:

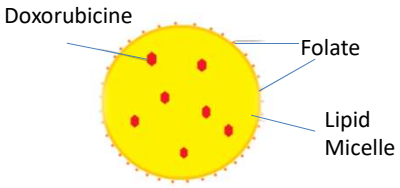
- Culture MCF-7 cells on our 3-dimensional scaffold
- Create LMNs with differing amounts of folate targeting molecules on the shell and doxorubicin in the core
- Treat MCF-7 scaffolds with various concentrations of targeted and untargeted LMNs and asses cell viability

Conclusions:

- Increased cytotoxicity of MCF-7 cell cultures when the doxorubicin loaded LMNs are functionalized with 30µg solution of folate versus the LMNs without folate.
- The LMNs functionalized in 300µg of folate did not show the same increase in cytotoxicity.
- One possible reason for this is that the extra folate hindered the attachment of the molecules to their receptors
- More trials will give a better understanding of the effectiveness of attaching folate at these different concentration.

Results:

A



B

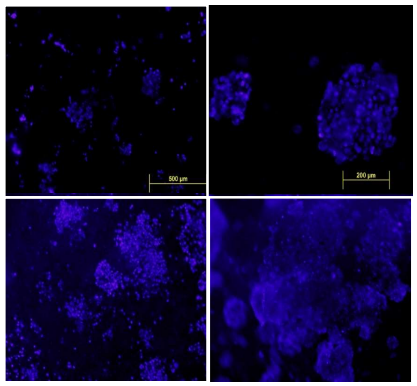
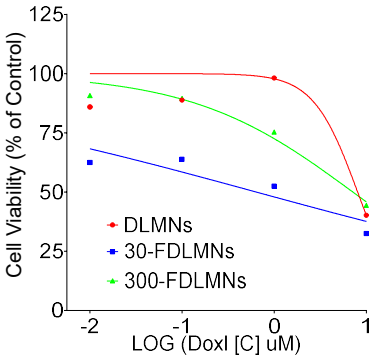


Figure 1: (A) Schematic of LMNs carrying Folate on the shell. **(B)** MCF-7 scaffold cell cultures at two different magnifications (100X-Left and 200X-Right).



	DLMNs	30-FDLMNs	300-FDLMNs
IC50	8.047	0.6372	7.141

Figure 2: Drug sensitivity in MCF7 cell lines. Cells were treated in triplicates with indicated concentrations of the identified drug for 48 hours. For cells grown on the scaffold drugs were added on day 5. Cell viability was determined using Cell Titer Glo assay (Promega). Average luminescence as a percentage of control in lapatinib treated cultures on the monolayer (Blue) and the scaffold (Red) is shown. Experiments were repeated at least two times. Graphs and statistical analysis were derived using GraphPad Prism software.

References:

1. Howell M, Mallela J, Wang C, et al. Manganese-loaded lipid-micellar theranostics for simultaneous drug and gene delivery to lungs. *Journal of controlled release : official journal of the Controlled Release Society*. 2013;167(2):210-218. doi:10.1016/j.jconrel.2013.01.029.