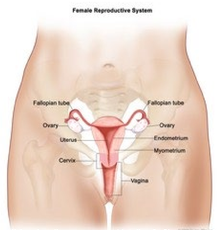
Liposomal Drug Delivery System for Treatment of Drug Resistant Ovarian Cancer

Samantha Gardner[1], Kharimat Lora Alatise[1], Emily Miller[1], Emily Grant[1], Angela A. Alexander-Bryant, PhD[1]

[1]Department of Bioengineering, Clemson University, Clemson, SC

INTRODUCTION

Ovarian cancer is the fifth leading cause of cancer mortality in women, with four out of five patients diagnosed in advanced stages, III and IV [1]. Late detection significantly reduces the survival rate from 90% in women with localized, early-stage diagnosis, to 30% when diagnosed in the late stages [2]. A debulking surgery prior to platinum-based chemotherapy is the current standard of care for treatment of ovarian cancer [2]. However, recurrence is a common problem, with nearly 75% of women who respond to initial platinum-based treatment experiencing relapse due to drug resistance [3]. Liposomes are a promising solution to overcoming drug resistance due to their biocompatibility and capacity to encapsulate both hydrophilic and hydrophobic drugs as well as complex small interfering RNA (siRNA). siRNAs are double-stranded RNA molecules that have the potential to downregulate the expression of genes related to drug resistance [4]. However, a drug delivery vehicle is necessary for the transport of siRNAs due to degradation of the molecules in a biological environment [4]. We aim to synthesize and characterize a liposomal system to deliver siRNA and paclitaxel to ovarian cancer cells.



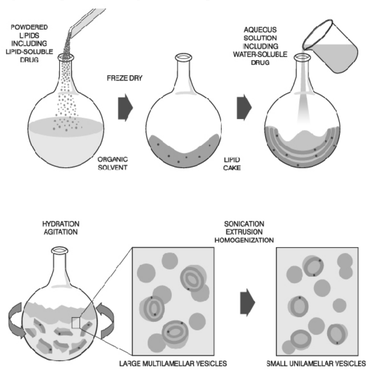
https://visualsonline.cancer.gov/details.cfm?imageid=8262

References

[1] Torre, L. A., *CA Cancer J Clin.* 2018;68(4):284-296, [2] Cortez, A. J., *Cancer Chemother Pharmacol.* 2018;81(1):17-38, [3] Norouzi-Barough L., *J Cell Physiol.* 2018;233(6):4546–4562, [4] Farra R., *Pharmaceutics.* 2019;11(10):547.

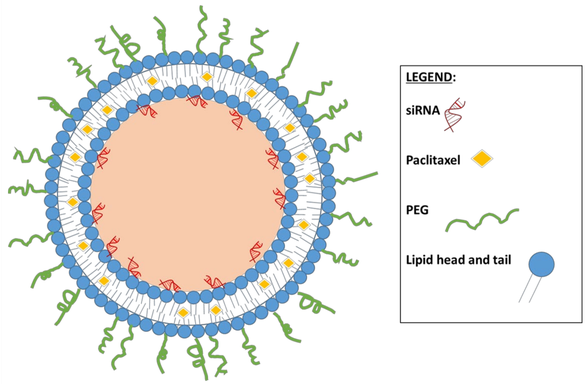
METHODS

Cholesterol (CHOL) and Cholesteryl hemisuccinate (CHEMS) liposomes were synthesized through lipid film hydration with paclitaxel and siRNA encapsulated in the hydrophobic and hydrophilic lipid layers, respectively.



https://www.sigmaaldrich.com/technical-documents/articles/biology/liposome-preparation.html

METHODS



Dynamic Light Scattering was used to characterize the liposomal system. The cytotoxicity of unloaded liposomes was evaluated through MTS assay on OVCAR3 and OVCAR3-T40, a wild-type and a paclitaxel-resistant human adenocarcinoma cell line. Uptake of liposomes into OVCAR3 and OVCAR3-T40 cell lines was examined using fluorescence microscopy.

RESULTS

1. Lipids formed uniformly sized liposomes that efficiently loaded siRNA.

Liposome Formulation	Size (nm)	PDI	Zeta Potential (mV)	siRNA Encapsulation Efficiency (%)
Blank CHOL	123.0 ± 2.49	0.164	32.3 ± 2.16	-
siRNA + PTX CHOL	114.9 ± 10.35	0.251	27.6 ± 1.79	99.8
siRNA + CHOL	100.5 ± 2.25	0.331	27.4 ± 1.40	-
Blank CHEMS	97.88 ± 2.39	0.219	29.0 ± 2.00	-
siRNA + PTX CHEMS	91.23 ± 7.66	0.214	23.2 ± 1.56	99.6
siRNA + CHEMS	80.78 ± 0.77	0.259	13.1 ± 1.66	-

Table 1. Characterization of CHOL and CHEMS Liposomes. Size, PDI and zeta potential were measured using DLS. siRNA encapsulation efficiency was determined using fluorescent siRNA.

RESULTS

2. Blank CHOL and CHEMS liposomes did not exhibit toxicity to OVCAR3 and OVCAR3-T40 cells at liposome concentrations less than 75 ug/mL.

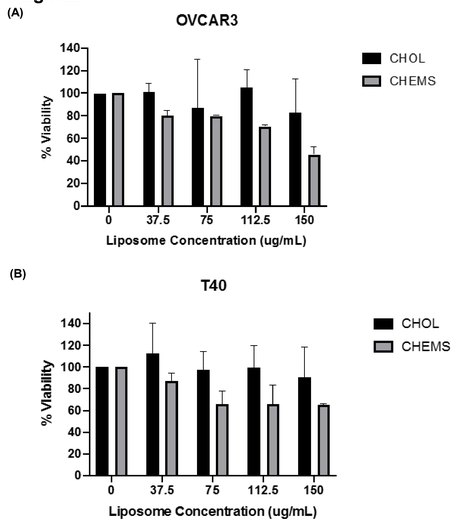
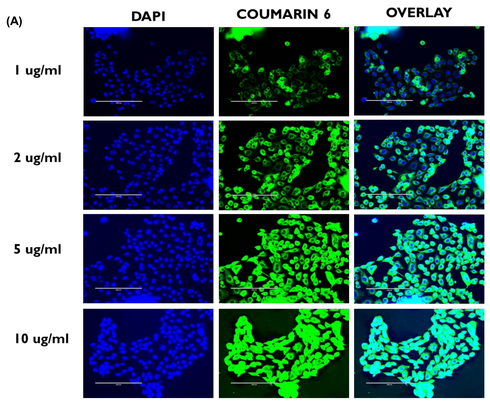


Figure 1. In vitro cytotoxicity of blank liposomes. Viability was assessed via MTS assay. (A) OVCAR3 cells were treated at various concentrations of CHOL and CHEMS blank liposomes for 24 hours. (B) OVCAR3-T40 cells were treated at various concentrations of CHOL and CHEMS blank liposomes for 24 hours.

3. Liposomes mediated uptake of Coumarin 6 into paclitaxel resistant cells.



RESULTS

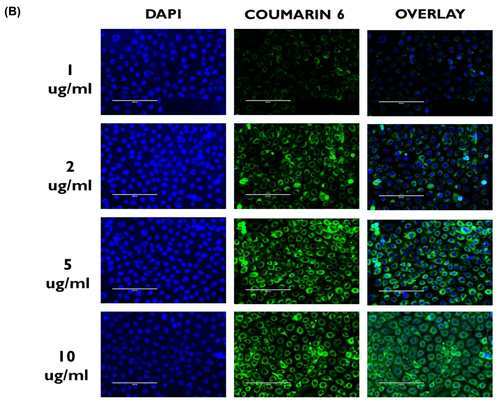


Figure 2. Cellular uptake of coumarin 6 loaded CHOL liposomes on OVCAR3-T40 cells. Fluorescent microscope images of differing concentrations of coumarin 6 loaded liposomes on sensitive and resistant ovarian cancer cells for 2 hours. Nuclei are stained with DAPI (blue). Coumarin 6 is green. (A) CHOL liposomes. (B) CHEMS liposomes.

CONCLUSIONS

- **Cationic CHOL and CHEMS liposomes were successfully formed with a monodisperse size and exhibited effective siRNA loading.**
- **Blank liposomes did not display any cytotoxic effects on sensitive and resistant ovarian cancer cells at concentrations of 75 ug/mL and below.**
- **CHOL and CHEMS liposomes mediated cellular uptake of fluorescent model drug, Coumarin 6.**

FUTURE WORK

- **Continue with cytotoxicity of blank liposomes experiments**
- **Examine expression of previously identified target genes**
- **Investigate efficacy of liposomal system in mediating gene silencing**
- **Viability studies on sensitive and resistant cell lines after treatment with liposomal system**
- **siRNA release studies on siRNA loaded liposomes**

ACKNOWLEDGEMENTS

Advisor: Dr. Angela Alexander-Bryant, Ph.D.
Lab Members: Timmy Samec, Megan Pitz, Kharimat Lora Alatise, Jessie Boulos, Alex Nukovic, Serena Gilmore, Anthony Hazleton, Aaron Williams, and Sarah Wilde

Thank you to George Duran from Stanford University for the donation of the resistant OVCAR3 cells.

Funding: This work was supported in part by the National Science Foundation EPSCoR Program under NSF Award # OIA-1655740. Any Opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect those of the National Science Foundation.