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Award Number: DAMD17-03-1-0603

TITLE: The Dynamics of Intracellular Uptake of Surface Modified Poly (d, L-Lactide-co-Glycolide) Nanoparticles in Breast Cancer Cells

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REPORT DATE: September 2004

TYPE OF REPORT: Revised Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2004	Revised Final	25 August 2003 – 24 August 2004

4. TITLE AND SUBTITLE

The Dynamics of Intracellular Uptake of Surface Modified Poly (d, L-Lactide-co-Glycolide) Nanoparticles in Breast Cancer Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-03-1-0603

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Hampton University
Hampton, VA 23668

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Internalization by cells of macromolecular drugs and drug loaded nanoparticles can be most advantageous in overcoming several limitations in cancer prevention and treatment. These systems are generally considered to be impermeable to cell membranes. However, internalization has been observed to occur via endocytosis. Affinity for cell membrane and high uptake is improved by several mechanisms including increased hydrophobicity, presence of surface charge or presence of targeting moieties in macromolecules or on nanoparticles. Specific targeting of deranged epithelial cells in the breast with biodegradable, controlled drug release nanoparticle systems is an attractive approach to solving problems or prevention and treatment of breast cancer. In this study, we sought to demonstrate that nanoparticles coated with an epidermal growth factor (EGF) would be preferentially internalized in MCF-7 breast cancer cells. Optimal nanoparticle sizes ranging in size of 120 – 200 nm were obtained using acetone and polyvinyl alcohol (PVA). Freeze dried nanoparticles were readily re-suspendable. MCF-7 cells were incubated with EGF-coated and non-coated nanoparticles. Observation cells by confocal microscopy after incubation with coated and non-coated nanoparticles failed to reveal nanoparticle internalization by MCF-7 cells.

15. SUBJECT TERMS
Nanoparticles, Intracellular Uptake, Cellular Drug Release

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

FORWARD

Dr. Lwandiko Masinde, Principal Investigator (PI), is no longer a Hampton University employee and neither he nor his lab notes can be located. The following is the University's best attempt to delineate his research findings. Specifically, the Office of the Executive Vice President working collaboratively with the Office of Sponsored Programs reviewed all grant office files, interviewed the current administrative team in the School of Pharmacy and reviewed the financial records for said grant from the business office files in an attempt to reconstruct the research record to determine research findings. Finally, an on-campus team of experts were empaneled to review all documents and/or materials associated with the grant and generate a technical report to close-out the work of Dr. Masinde. The team consisted of Dr. Eric Sheppard, Dean of the School of Engineering; Dr. Isai Urasa, Chair of the Department of Chemistry; and Dr. Jale Akyurtlu, Professor of Chemical Engineering.

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STATEMENT OF PROBLEM STUDIED

Introduction:

Reduced toxicity and improved efficacy of many established drugs can be achieved by direct intracellular delivery using nanoparticle systems. For the new drug classes such as peptides and proteins, facilitated cellular uptake of nanoparticle drug carrier systems can offer targeted cellular drug release with protection of drug from degradation (1). Drug resistance mechanisms dependent on p-glycoprotein drug efflux can be improved by intracellular drug delivery and action. A unique possibility is presented, when abherant epithelial cells in the breast are targeted using polymeric nanoparticles loaded with cancer preventing and/or treating drugs. Less information has been revealed about the behavior of breast cancer cells with PLGA. Surface modified nanoparticles using EGF are designed to influence PLGA nanoparticles cell uptake, since it is widely known that EGF undergoes receptor-mediated endocytosis, hence bringing about ligand-receptor complex internalization (2). The purpose of the study was to develop nanoparticle sizes of PLGA, possessing superior properties for breast cancer cell targeting; relying on the PLGA-EGF complex interaction with (EGF) receptors which are known to be overexpressed in most cancer cells and in some breast cancer cells. EGF is widely investigated as a tumor seeking agent.

Body:

The study sought to establish that MCF-7 cells were capable of internalizing PLGA nanoparticles, and that this process would be enhanced by the presence of an EGF on the surface of nanoparticles. Breast cancer cells were incubated with uncoated and EGF-coated nanoparticles. The coating was undertaken by equilibration using several aqueous concentrations of EGF at room temperature over a 24 hour period with slow stirring. It was not possible to evaluate the nature of the coating by, for example, determining change in the zeta potential or physical surface structural change by electron microscopy. However, there was some slight increase in the mean nanoparticle diameters show by photon correlation method. This slight change could have resulted from swelling by hydration. It is, therefore, not established by this study, whether failure to internalize nanoparticles by breast cancer cells is due to the lack of interaction between the nanoparticle-coated EGF and the EGFReceptor on the cell,

or the coat was not adequate structurally. A more precise approach to the investigation of the effect of EGF in the intracellular uptake of PLGA is to synthesize PLGA polymers linearly linked with RGF. These complexes would then be used to form nanoparticles to be used for cell uptake. This part of the study, together with that involving synthesis of the PLGA-PEG diblock copolymer (PLGA-polyethylene glycol) for subsequent attachment of EGF was not carried out as envisioned. Efficient vacuum generation required in synthesis, and the high temperatures, plus the difficulty of characterizing the products precluded carrying out these experiments. Fluorescein-loaded nanoparticles were prepared to investigate in vitro release, and to determine an increased amount intracellularly after incubation with cells. Thorough washing followed by digestion of cells after incubation over a 24 hour period failed to reveal that there was an increased amount fluorescein in cells as a result of nanoparticle internalization. Confocal microscopy observation failed to show nanoparticle internalization by MCF-7 cells. Similar nanoparticle systems were analyzed by in vitro fluorescein release. A sustained release of fluorescein from nanoparticles was observed over a 48-hour period, releasing about 80 percent of the payload in this time period.

SUMMARY OF RESULTS:

- Nanoparticles were prepared using the emulsification/solvent evaporation method in accordance with the approved Statement of Work based on the proposal; as part of this process, some nanoparticle samples were loaded with tetramethylrhodamine-labeled dextran for intracellular localization fluorescence study: The PI was successful in producing a sufficient sample of the nanoparticles to proceed to the evaluation phase, despite an issue with generating the required vacuum.

- Evaluation of the nature of the coating was attempted by measuring the change in the zeta potential: the results were inconclusive, as the PI had difficulties characterizing the products.

- Evaluation of the physical surface structural change was attempted by electron microscopy: the results were inconclusive, as the PI had difficulties characterizing the products.

- Evaluation of the mean particle diameters by the photon correlation method: This characterization method was successful and yielded results: a size distribution analysis was carried out, and a slight increase in the mean nanoparticle diameter was observed, though the PI noted that this change may have been due to swelling by hydration.

- Similar nanoparticle systems were analyzed by in vitro fluorescein release: a sustained release of fluorescein was observed from these particles over a 48-hour period, releasing about 80% of the payload in this time period.

Key accomplishments:

- Nanoparticles efficiently prepared and size analysis
- Drug loaded nanoparticles, and in vitro release study
- MCF-7 cell viability in presence of nanoparticles

Reportable Outcomes:

There are no reportable outcomes at this time.

CONCLUSIONS:

The negative results observed from this study are not conclusive with respect to the probable uptake of PLGA nanoparticles by MCF-7 cells. Improved support facilities that offer more refined analysis of experimental outcomes are needed to successfully run these studies.

Challenges to be addressed if this work is continued:

- The PI was unable to generate and maintain the vacuum required for a higher scale of synthesis.
- The PI was unable to maintain the proper high temperatures required for many of the procedures.

- The PI faced difficulties in characterizing the products via electron microscopy and zeta potential measurements.

REFERENCES:

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2. D.C. Drummond, K. Hong, J.W. Park, C.C. Benz, and D.B. Liposome. Targeting to Tumors Using Vitamin and Growth Factor Receptors. *Vitam. Horm*.60: 285 – 332 (2000).

APPENDICES:

None