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TITLE: Nanoparticle: Monoclonal Antibody Conjugates: A  
Novel Drug Delivery System in Human Breast Cancer

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**13. ABSTRACT (Maximum 200 Words)**  
*The work proposed in this grant involves the development of nanoparticle: monoclonal antibody conjugated for use as a therapeutic approach to targeting specific breast cancer cells. As described in the accompanying document, we have successfully produced these nanoparticle: antibody conjugates, and have examined them with regard to drug release. We have also examined them with regard to their ability to bind to HER-2 positive breast cancer cells. Our current assays of this binding suggest that binding is non-specific. It is felt that this non-specificity may represent a failure of the assay utilized to examine binding, and another assay is currently being developed. In addition, we are currently examining other nanoparticle substrates that would have preferential drug release qualities. Early studies suggest that the development of these is a feasible goal.*

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## Introduction

The toxicity of systemic cytotoxic therapy remains a significant clinical problem, as does its lack of specificity. Delivery of cytotoxic agents to cancer cells is essentially a random function, resulting in variable tumor tissue levels and "innocent bystander" toxicity in normal tissues. A novel and previously unutilized technology promises to overcome the problems associated with liposomal technology, while fulfilling its promise. The research discussed here utilizes biodegradable nanoparticles of poly(lactic-co-glycolic acid) with free pendant chains of poly(ethylene glycol) onto which antibodies are conjugated to yield targeted, biodegradable, nanoparticulate drug delivery systems with delivery times of weeks to months. We currently are evaluating this previously unexplored technology, combined with a tumor-specific antibody (Herceptin), to increase the tumor-specific cytotoxicity and specificity of cytotoxic agents, both in vitro and in vivo.

## Body

*Task 1:* To develop techniques to successfully encapsulate taxol and doxorubicin into biodegradable nanoparticles of poly(lactic-co-glycolic) acid (PLA/PGA) with poly(ethylene glycol) (PEG) present at the surface of the nanoparticles to allow for nanoparticle targeting. (months 1-12)

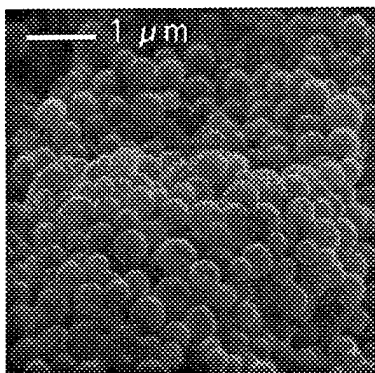
*Task 2:* To prepare antibody-PEG and antibody-PLA/PGA-g-PEG conjugates (months 6-10)

*Task 3:* To develop techniques to successfully encapsulate taxol and doxorubicin into biodegradable nanoparticles of poly(lactic-co-glycolic) acid (PLA/PGA) with poly(ethylene glycol) (PEG)-antibody conjugates present at the surface of the nanoparticles to allow for nanoparticle targeting. (months 12-24)

Much of tasks 1-3 were accomplished during the first year of the study, as documented in our previous report. This work continued in the second year of funding. The work of that year and the second year with regard to tasks 1-3 are summarized as follows:

### Nanoparticle Preparation

Biodegradable PLAGA (65:35 lactide:glycolide, MW 33,000) was purchased from Birmingham polymers (Birmingham, AL). Acetone, taxol and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). Doxorubicin was provided by Pharmacia-Upjohn and the herceptin was provided by Indiana University School of Medicine. For the general nanoparticle preparation process, 100 mg of PLAGA was dissolved in 3 ml of acetone in a 20 ml scintillation vial. To this solution was added 10 ml of BSA solution (1.0 mg/ml) and the resulting emulsion was very briefly shaken by hand before being immersed in a bath-type sonicator (Aquasonic, model 50D) operating at 45W for 1-minute. The emulsion was then transferred to a vacuum Erlenmeyer (150 ml) with an additional 10 ml of water used as a rinse. The emulsion was stirred at 400 RPM while under a modest vacuum (~100 mm Hg, with bleeding) for 30-45 minutes to remove residual solvent. After the organic solvent was completely removed, a small volume of the emulsion was removed (while stirring continued) for sizing using a Coulter Nano-Sizer calibrated with 200 nm latex spheres (Polysciences, Warrington, PA). The solution was centrifuged and washed three times with progressively more dilute BSA solutions to remove unencapsulated drug and unbound antibody.

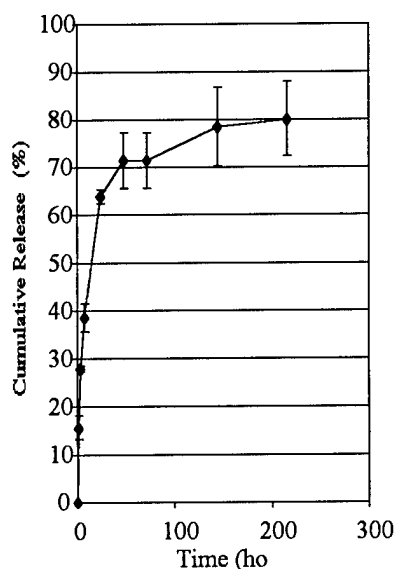


A scanning electron micrograph of PLAGA nanoparticles containing doxorubicin and with herceptin attached is shown here.

Modifications to this method to achieve incorporation of taxol and doxorubicin as well as attachment of the targeting antibody will be discussed.

### Drug Delivery

Release studies were carried out using equilibrium dialysis. Dialysis cells with 1 ml capacity on both the donor and receptor sides were prepared with approximately 50 mg of freeze-dried formulation material (surfactant and particles) suspended in 3 ml of buffered saline. The receptor side was separated from the suspension side by dialysis membrane of 50K molecular weight cutoff. Sample solutions were removed and replaced with fresh saline at each sampling time. The amount of taxol was measured using HPLC and the amount of doxorubicin was measured using UV-Vis Spectrophotometry. Release profiles were measured in triplicate and corrected for the volume of the suspension side of the dialysis cell. A representative release profile of doxorubicin from PLAGA nanoparticles with an average particle size of



312 nm is shown below.

Task 4: To examine the in vitro and in vivo efficacy of the NP:MAB conjugates (months 18-36).

Task 4 was initiated during the past 12 months. Our initial goal has been to examine the ability of NP-MAB conjugates to bind to HER-2/neu-expressing cancer cells in vitro by flow cytometry. This was initially performed through the following experiment: freshly prepared nanoparticles containing doxorubicin were either conjugated to Herceptin monoclonal antibody or not, then mixed with breast

cancer cells that are or are not positive for HER-2 cell surface expression. The results of multiple flow cytometric analyses suggested that this approach was not technically feasible, in that fluorescence intensity was similar for all tested cells.

The explanation for this failure presumably represents the early release of doxorubicin from nanoparticles, uptake by breast cancer cells, and fluorescence of the breast cancer cells. We are currently examining another approach for evaluation of NP:Mab binding to breast cancer cells, utilizing a secondary, fluoresceinated anti-idiotypic antibody directed against Herceptin. Results from this analysis should become available in the near future.

We have prepared a total of 53 formulations using doxorubicin or epirubicin from nearly every PLGA available (Birmingham Polymers and Alkermes). All but two formulations used 100 mg of PLGA, with 2 lots prepared at 1 gram of PLGA. The Alkermes PLGA lot 9007-394 gave the overall best performance based on encapsulation, drug burst, length of release, and total amount drug released. PLGA lot 9007-394 is acid-capped 50:50 PLGA with a MW of about 11 KDa.

PLGA lots from BPI tested were D97044 (65:35, 33000 MW), D97121 (65:35, 50000 MW) and D95061 (70:30 grafted to 5000 MW PEG). We also performed chemical modifications of D95061 and used the resultant polymer in a few formulations. Most of these formulations used acetone as the organic phase solvent and albumin as the aqueous phase surfactant. In general, formulations prepared from these polymers did not perform well. Specifically, the encapsulation efficiency and release profiles were the most disappointing. Encapsulation efficiencies were in the 10-30% range and the release profiles showed a larger burst and a total release time of only 24 hours.

PLGA lots from Alkermes consisted of acid-capped and ester-capped 50:50's with varying molecular weights. Using ester capped PLGA's from Alkermes resulted in similar results to the BPI polymers (lower encapsulation efficiency, shorter release, larger burst, less drug released etc.) The acid capped polymers performed extremely well with regard to encapsulation efficiency of epirubicin and doxorubicin (nearly 100% regardless of PLGA MW). However, the acid-capped PLGA with a MW of 11000 (9007-394) clearly performed the best in terms of nanoparticle yield, re-suspendability and total drug released in vitro. We varied the drug loadings between 2 and 10 % by mass using 9007-394. All these formulations outperformed the ester-capped and BPI np's, however, the nanoparticles with the lowest amount of drug had the best looking release in vitro profile in terms of burst and total amount of encapsulated drug released.

SSA-PEG and the antibody have been included in some formulations as well. The presence of SSA-PEG was verified using 1-gram formulations such that sufficient sample could be tested. Only those particles without doxorubicin or epirubicin could be assayed for SSA-PEG as the drug greatly interferes with the assay. An attempt to assay for residual antibody on the nanoparticles was inconclusive.

In summary, this project is at a point where we can prepare at least up to 1-gram of PLGA nanoparticles containing doxorubicin or epirubicin and SSA-PEG in significant amounts. If 100 mg formulations are prepared, acetone and BSA should be used as the surfactant/solvent combination (with methanol for drug). If one gram formulations are prepared then it is better to use ethyl acetate and BSA with methanol for the drug and PEG.

### Key Research Accomplishments:

We have formulated nanoparticle:monoclonal antibody conjugates that should, in the near future, be ready for in vivo animal experimental use.

### Reportable Outcomes

None at Present

### Conclusions

As above, we continue to examine nanoparticle substrates that offer the best chances for biologic effectiveness as anti-tumor agents. It remains our purpose to develop these for preclinical and clinical studies.

### References

None

### Appendices

None