POLY(LACTIDE-CO-GLYCOLIDE) NANOPARTICLES FOR CONTROLLED DELIVERY OF DOXORUBICIN FOR THE TREATMENT OF CANCER

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INTRODUCTION

Current cancer treatment methods have a number of limitations that lead to poor therapeutic results. Chemotherapeutic treatments lack specificity and lead to the damage of healthy tissue, especially of the normally dividing cells of the bone marrow, skin, and gastro-intestinal mucosa, among other tissues (Twardowski, 2002). Some chemotherapeutic agents, including the one here utilized, can extravasate into the perivascular spaces and cause severe localized tissue damage or necrosis because of their vesicant properties. In addition, neoplastic cells readily mutate and many cancers have developed resistance to chemotherapeutic agents. The need for improved therapies for the treatment of cancer is great, and one of the ways to overcome the deficiencies of current treatment methods is to develop delivery systems that significantly improve the pharmacological characteristics of the drug *in vivo.*

The drug delivery system here utilized consists of biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles. These nanoparticles are used as vehicles for the targeted and controlled delivery of doxorubicin (DOX), a commonly used and potent chemotherapeutic agent of the anthracycline antibiotic family. Clinical use of DOX is currently limited to a maximum lifetime dose of 550 mg/m^2 because of known cardiotoxicity associated with anthracycline treatment (Lum, 1985). PLGA nanoparticles will protect the patient from toxic effects associated with high concentration bolus doses, and release the drug in a controlled manner so that its concentration is maintained within therapeutic levels for longer periods of time. In addition, these nanoparticles are small enough to circulate through capillaries, cross the highly-permeable vasculature supplying blood to tumors because of the enhanced permeability and retention effect (EPR), and enter tumor cells through endocytosis. PLGA nanoparticles can be potentially targeted to specific tissues by including targeting moieties in the formulation, and modified to include poly(ethylene glycol) pendant chains for increased circulation time in the vasculature. These favorable pharmacological characteristics result in improved therapeutic efficacy, better use of the pharmaceutical agent, and increased patient compliance and quality of life.

In this work we report on the preparation, characterization, and *in vitro* evaluation of PLGA nanoparticles loaded with doxorubicin. These particles were prepared through a precipitation technique followed by solvent evaporation. Nanoparticles were characterized with respect to size, morphology, zeta potential, loading, encapsulation efficiency, and release profile. Cytotoxicity and cellular uptake of these nanoparticles were studied *in vitro* in MDA-MB-231 breast cancer cells.

EXPERIMENTAL METHODS

Blank and doxorubicin-loaded nanoparticles were prepared through a modified precipitation, solvent evaporation method. Approximately 6mg of doxorubicin (Fisher Scientific, Hampton, NH) were dissolved in methanol, and mixed with a solution of 100mg of 50:50 poly(lactide-co-glycolide) (Medisorb, Cincinnati, OH) in acetone to form the organic phase. This phase was then added to an aqueous solution containing bovine serum albumin. After brief sonication, the suspension was stirred under vacuum for 45 minutes to remove the solvent. Nanoparticles were recovered through centrifugation, washed, and lyophilized. Particle morphology was studied with scanning electron microscopy. Particle size was determined with a Coulter Nanosizer. Encapsulation efficiency and loading were determined from the supernatants generated during the recovery step of nanoparticle preparation, and by dissolving a known mass of nanoparticles in a solution of dichloromethane/methanol (60/40 v/v%, respectively). The surface charge of these nanoparticles was assessed through zeta potential measurements with a ZetaPlus (Brookhaven Instruments Corporation). Release studies were performed *in vitro* in buffered and non-buffered saline at 37^{o}C to determine the release kinetics of these particles. UV/Vis absorbance was utilized to determine the concentration of doxorubicin in solutions. MDA-MB-231 breast cancer cells were used to determine cellular uptake of the nanoparticles through confocal microscopy. The cells were incubated with suspensions of nanoparticles or solutions of free drug for specific time periods, washed and fixed before observation. The cytotoxicity induced by the doxorubicin-loaded nanoparticles was determined with an MTT-based assay, and compared to the effects of free drug, and blank nanoparticles. The cells were exposed to suspensions of nanoparticles or free drug for 2 hours, after which the media was replaced with complete cell media and the cells were incubated for 3 days prior to viability determination.

RESULTS AND DISCUSSION

Nanoparticles were found to be roughly spherical, as seen in the scanning electron microscopy image in Figure 1. The average nanoparticle diameter was determined to be 230 nm and was found to be independent of drug loading. As observed, although not of monodispersed size, the particle size distribution is quite reasonable. The average zeta potential was determined to be approximately -45 mV. The maximum loading achieved was close to 5 mg of doxorubicin per 100 mg of nanoparticles (5%), and the average encapsulation efficiency was greater than 60% for all batches.

As seen in Figure 2, there were significant differences between the doxorubicin release characteristics of nanoparticles suspended in buffered and non-buffered saline, as expected. The release of nanoparticles in non-buffered saline occurred at a much faster initial rate possibly because of the autocatalytic degradation of PLGA in the acidic environment produced by the degradation products of PLGA. In buffered saline doxorubicin was released at an almost constant rate during the first week, and no burst release was observed. Consequently, this system would be able to deliver this chemotherapeutic agent in a controlled manner over an extended period of time, thus maintaining the therapeutic effect longer and eliminating the toxic effects that high-concentration bolus doses cause, ultimately improving the quality of life of the patient.

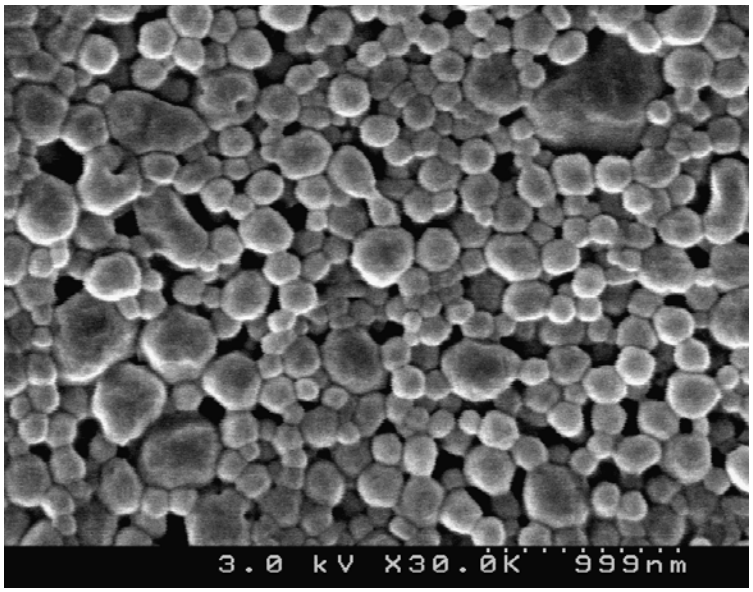

Figure 1. Scanning electron microscopy image of doxorubicin-loaded poly(lactide-co-glycolide) nanoparticles.

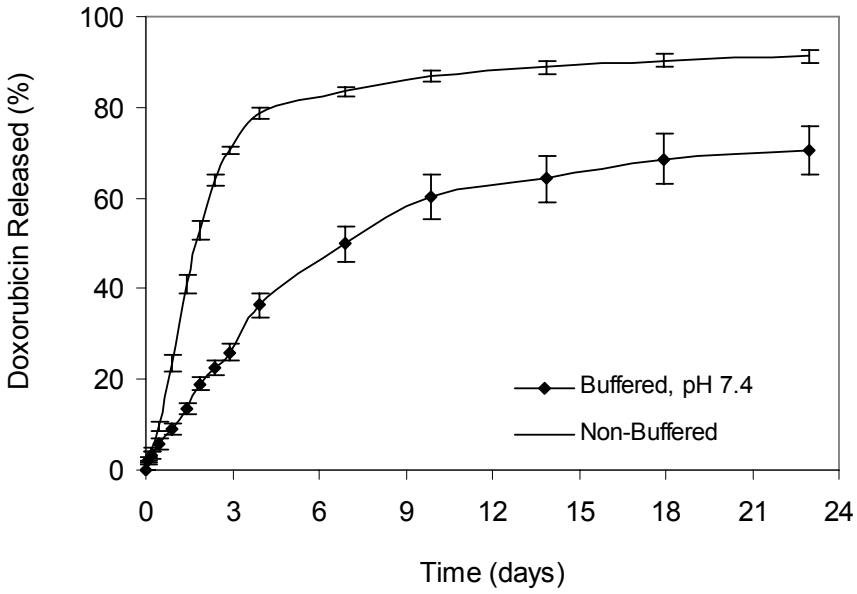

Figure 2. *In vitro* release of doxorubicin from poly(lactide-co-glycolide) nanoparticles in buffered and non-buffered saline. Values represent release data from 5 batches of nanoparticles, each batch in triplicate. Error bars represent the standard deviation of all data.

Cytotoxicity studies revealed that free doxorubicin and doxorubicin-loaded nanoparticles significantly reduced viability compared to control and to blank nanoparticles. Blank nanoparticles only significantly reduced cell viability at the highest concentration. Cytotoxicity induced by doxorubicin encapsulated within the nanoparticles did not seem to

increase compared to free drug. Despite the lack of significant increase in cyctotoxicity by the nanoparticles, this formulation (1) maintains the activity of the drug, (2) is readily endocytosed despite the macromolecular size, and (3) is at least as effective as the free drug.

Cellular studies revealed that the nanoparticles are readily uptaken into the cells. High fluorescence intensity was observed even after only 1 hour of incubation with nanoparticles or free drug. An increase in fluorescence intensity was observed with increased concentration of doxorubicin-loaded nanoparticles or free doxorubicin, and with increased time of exposure, as expected. For all times of incubation, and as shown in Figure 3, the fluorescence intensity of cells exposed to doxorubicin-loaded nanoparticles was significantly higher than cells exposed to free doxorubicin.

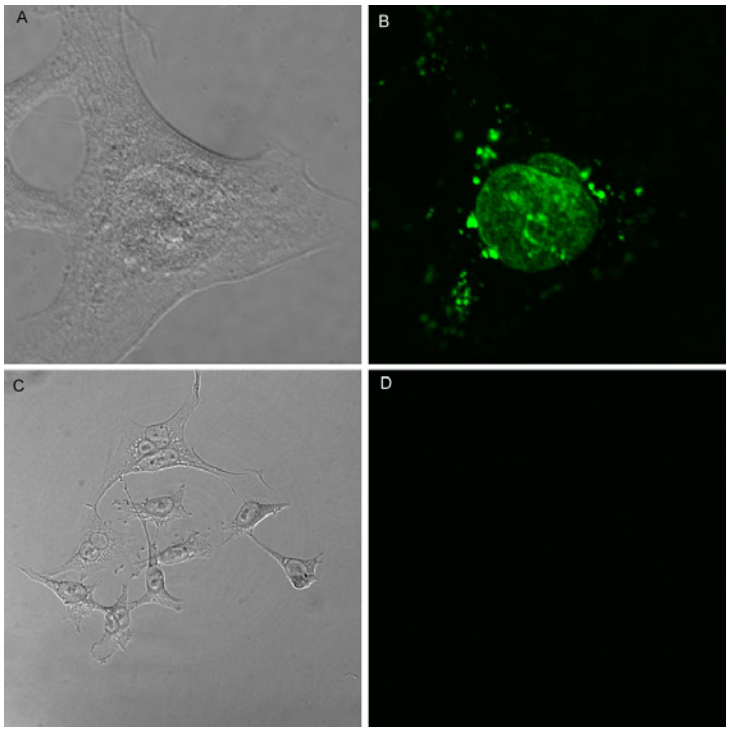

Figure 4. Transmission (A) and fluorescence confocal (B) image of breast cancer cells after exposure to doxorubicin loaded PLGA nanoparticles at a concentration equivalent to 10 µg/ml of doxorubicin for 2 hours. Brightfield (C) and corresponding fluorescence (D) image of control cells incubated in PBS at a lower magnification.

CONCLUSIONS

PLGA nanoparticles were successfully formulated, characterized and evaluated *in vitro*. These nanoparticles promise to be an effective system for targeted and controlled release of doxorubicin or other chemotherapeutic agents with reduced systemic toxicity, increased therapeutic efficiency, and increased patient compliance.

ACKNOWLEDGEMENTS

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