A Release Rate Study for Controlled Drug Delivery Using Niosomes

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ABSTRACT

The emerging technology of drug-encapsulated nanoparticles plays a critical role in the development of treatment for various life-threatening diseases due to the versatility, control, and future potential of the design of these systems. A current treatment technique for the treatment of brain tumor cells, after major surgery, utilizes a drug adhered to a biodegradable polymer wafer that provides the release of medication over about 120 hours. The setback to a design such as this is the levels of toxicity that can be delivered to other organs in the body due to over-medication of the drug, causing adverse reactions in the patients. This project proposes to design a drug-delivery vehicle that utilizes a double controlled mechanism that is based on a package within a package system, or a smart-packaging system. The drug will be encapsulated in non-ionic surfactant vesicles, or niosomes, and then embedded in a biodegradable temperature- and pH-sensitive chitosan polymer hydrogel. This double packaging system will allow for the controlled release of the drug based on the diffusion properties and physical characteristics of the niosomes and chitosan hydrogel. The goal of this investigation was to study the release rate of the drug from the niosome in an *in vitro* environment. The niosomes were prepared by thin film hydration and sonication, and 5(6)-carboxyfluorescein dye was encapsulated in the vesicles to mimic the behavior of the drug. The concentration of the dye varied from 4mM to 5mM to 14mM. The niosome solution was placed in a semi-permeable cellulose membrane and submerged in a bulk solution of either Milli-Q water or PBS. The system was placed on a stirring plate and allowed to run for 72 hours. Samples were taken from the contents in the membrane and contents in the bulk solution at different time intervals. Fluorescence spectroscopy was used to measure the intensity and concentration of the samples. It was found that the dye was released from the niosomes submerged in the water solution within the first 10 hours of the experiment, indicating their instability in that environment. It was also found that the niosomes submerged in the PBS solution maintained their stability, with their release rates being relatively lower than in the water solution. It was shown that the release rate behavior of the niosomes was similar regardless of initial concentration of dye encapsulated in the vesicles. It can be concluded that the niosomes are unstable in Milli-Q water and require a hydrogel that will stabilize the vesicles, similar to PBS. The hydrogel will prevent the premature release of the drug from the vesicles. This technique allows for control over the release rates of the drugs, which can decrease toxicity to other parts of the body, increase direct utilization of the drug, and increase the survival time of brain cancer patients. It is the goal of this research to design a novel drug-delivery technique that will be safe and effective for the treatment for brain tumor cells, while improving the quality of life and duration of survival for these patients.

INTRODUCTION

There are several methods that have attempted to improve upon traditional chemotherapy treatment for brain tumors using local delivery of Temozolomide. This is a drug used to treat brain tumors in adults [1]. One of the current treatments is the use of Gliadel wafers that are FDA approved [2]. The wafers are implanted into the brain cavity and release

the drug over a two week time period as the polymer slowly degraded [3, 4]. In this project, a combined system formed by niosomes (non-ionic surfactants) and hydrogels are investigated to increase the rate of release and improve its efficiency to target brain tumors. The niosome/hydrogel two-control mechanisms allow precise dosage and rate of release of therapeutic drugs from coupling the diffusion out of niosomes and through the polymer network of the hydrogel. Advantages to this system are that niosomes do not interfere with the performance of the drug and the moldable hydrogel can fill the shape of the entire cavity. A chitosan polymer can be used and be made to respond to changes in environmental conditions such as temperature and pH [5, 6]. This paper presents the performance of the niosomes to release an encapsulated compound through a cellulose membrane, in order to mimic the effect of a simple layer of hydrogel.

EXPERIMENT

The experimental procedure consisted of a series of diffusion experiments [7]. Briefly, a 2 ml volume of a dye-encapsulated niosome solution was placed in dialysis cellulose membrane. A 5(6)-carboxyfluorescein dye was encapsulated into the niosomes. Protocols to prepare niosomes have been discussed elsewhere [8]. The membrane was submerged in 85 ml of either Milli-Q water or PBS solution. The system was placed on a stirring plate and allowed to run for 3, 6, 9, 12, 24, 72, or 144 hours. The experiments were stopped at the designated time and samples were taken from inside the dialysis tubing and from the bulk solution. Samples from inside of the dialysis tubing contained dye that was released from the niosomes as well as intact niosomes in which dye had not been released. The amount of dye that remained in the niosomes was measured by using a detergent, Triton-X 100, to force the niosomes to burst and release their dye [8]. The concentration that was encapsulated in the intact niosomes could then be determined by taking the difference between the “naturally” released dye and the detergent-forced released dye. We measured the dye release with a fluorescence spectrometer. The exact concentration of released dye was calculated from comparison to calibration standards. Experiments were performed with 4mM, 5mM, and 14mM in a Milli-Q bulk solution, and one experiment was performed with 5mM in a PBS solution.

RESULTS AND DISCUSSION

Our experiments show that we are able to measure the amount of drug or dye that is being release per time as a function of the concentrations and the concentration of media outside the membrane. The dye used inside the niosomes was chosen because its molecular weight is similar to Temozolomide. The Gliadel wafers release their drug over a 2-3 week time period [9]. The times selected for our experiments were based on about a 1 week time frame, initially. Based on the behavior of the system, it was observed that most of the release activity was occurring during the first 24 hours, so it was determined to monitor the experiments at periods within that time. Two important findings were determined. One, the number of moles released from inside the niosomes is a function of the initial concentration of the encapsulated dye. This means that higher release rates were measured at higher concentrations and vice versa. The relation, however, is not a straight line and it seems to be a function of the media to which the niosomes are being exposed and the saturation state of the solution outside the membrane. Second, the release of the dye occurred within 11 +/- 1 hours when the niosomes were exposed to Milli-Q water. But, the release rate was much slower when the niosomes

were exposed to PBS. Figure 1 shows a comparison between the free moles of dye in the membrane when the niosomes have been exposed to Milli-Q water and PBS. This is an illustration of the release rate of the dye from the niosomes. In this case, the niosomes were encapsulated with a 5mM concentration of dye.

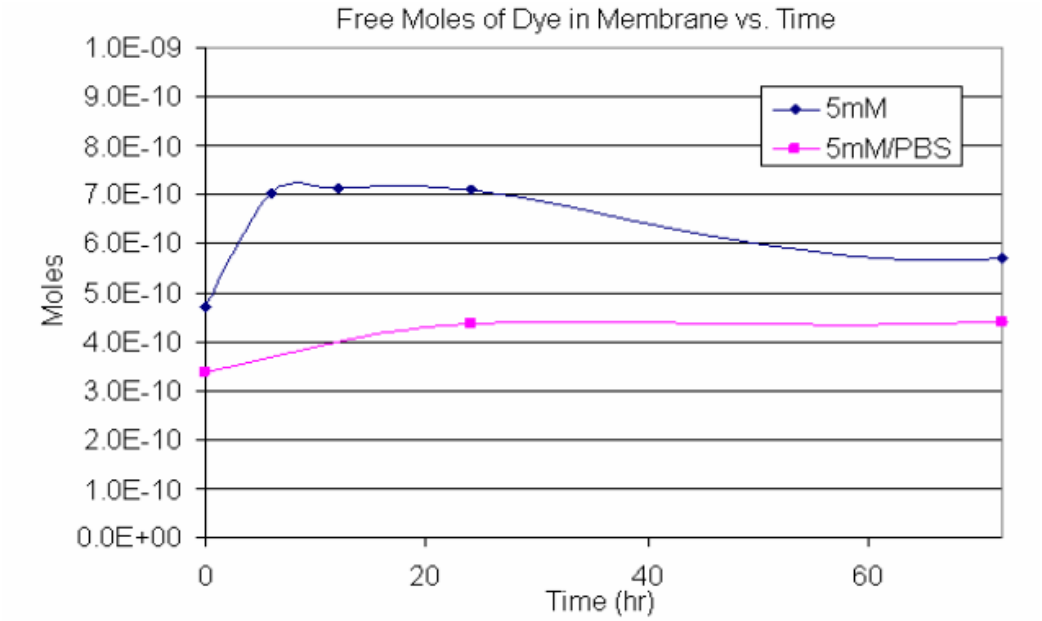

Figure 1. The moles of free dye inside the cellulose membrane. The results for the 5mM initial concentration of dye encapsulated niosomes exposed to Milli-Q water (●) and PBS (■) are represented. These moles represent the moles of dye that were released from the niosomes, and indicates that the niosomes in the PBS are stabilized.

CONCLUSIONS

The results indicate two interesting observations of the system. The release rate of the dye from the niosomes is independent of the concentration of initial dye encapsulated in the vesicles. The second observation is that the vesicles remain in tact for a longer period of time when placed in contact with a PBS solution than when placed in contact with an aqueous water solution. The niosomes are prepared in a PBS solution, so when the osmotic pressure changes, such as with water, the niosomes will tend to be less stable and the maximum release rate will occur within 11 +/- 1 hours.

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