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Simultaneous drug release at different rates from biodegradable polyurethane foams

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Abstract

In this study, we present an approach for the simultaneous release of multiple drug compounds at different rates from single-phase polyurethane foams constructed from lysine diisocyanate (LDI) and glycerol. The anti-cancer compounds DB-67 and doxorubicin were covalently incorporated into polyurethane foams, whereby drug release can then occur in concert with material degradation. To begin, the reactions of DB-67 and doxorubicin with LDI in the presence of a tertiary amine catalyst were monitored with infrared spectroscopy; each compound formed urethane linkages with LDI. Fluorescent spectra of DB-67 and doxorubicin were then recorded in phosphate-buffered saline, pH 7.4 (PBS), to ensure that each anti-cancer compound could be quantitatively detected alone and in combination. Doxorubicin and DB-67 were then incorporated into a series of degradable LDI–glycerol polyurethane foams alone and in combination with one another. The sol content, average porosity and drug distribution throughout each foam sample was measured and found to be similar amongst all foam samples. The stability of DB-67 and doxorubicin's fluorescent signal was then assessed over a 2-week period at 70 °C. Release rates of the compounds from the foams were assessed over a 10-week period at 4, 22, 37 and 70 °C by way of fluorescence spectroscopy. Release was found to be temperature-dependent, with rates related to the chemical structure of the incorporated drug. This study demonstrates that differential release of covalently bound drugs is possible from simple single-phase, degradable polyurethane foams.

Keywords: Polyurethane; Drug delivery; Doxorubicin; DB-67; Lysine diisocyanate

1. Introduction

DB-67 (7-*tert*-butyldimethylsilyl-10-hydroxycamptothecin) and doxorubicin (adriamycin) are potent anti-cancer compounds, each exerting their cytotoxic effects by interrupting processes essential to cellular growth and proliferation. DB-67 functions through inhibition of topoisomerase I, a member of a nuclear enzyme family responsible for repairing DNA during replication [1–4]. Doxorubicin functions via two mechanisms: by intercalating DNA and inhibiting its transcription, and by inhibiting the enzyme topoisomerase II [5–8]. One mechanism utilized by tumor cells to evade the cytotoxic actions of topoisomerase inhibitors involves switching between various enzyme isoforms [9]. It may therefore prove beneficial to construct materials capable of releasing both DB-67 and doxorubicin in a staggered manner to subvert the natural resistance response to these agents.

When using chemotherapeutics to combat malignancies in the body, it is impossible to avoid exposing normal functioning cells to their deleterious effects; the toxic side effects of these compounds ultimately are responsible for limiting their clinical efficacy [10–14]. Many strategies have evolved attempting to selectively deliver cytotoxic chemotherapeutics to localized regions of malignant tissue within the body

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[15,16]. One such strategy that has proven effective is the implantation of degradable polymeric devices at the tumor site loaded with select chemotherapeutic agents [17,18]. Instead of administering a drug to the entire body, these strategies serve to limit the exposure of the therapeutic to only a single body compartment or even to selected regions of a single compartment. This strategy has proven efficacious and has resulted in several patented devices approved by the FDA for the treatment of solid tumors [19–23]. Most often, these systems focus on the delivery of a single therapeutic, relying solely on diffusion as the primary release strategy. In these systems, the drug load will often be exhausted prior to the degradation and absorption of the polymeric material.

Systems for the simultaneous delivery of multiple therapeutic compounds have been proposed [24–28]. Polymeric systems for the delivery of multiple agents generally consist of numerous distinctly defined phases each capable of delivering a separate compound [29,30]. Typically, each phase possesses different diffusive properties that govern the liberation of the impregnated compound. Some of these systems even use multiple synthetic layers to achieve staggered rates of delivery [31]. However, the synthesis of such systems is often complex, costly and time-consuming, ultimately preventing their commercialization and application in a clinical setting. A single-phase polymer capable of simultaneous release of multiple therapeutic compounds at differing rates would offer an advantage over these systems in terms of cost and ease of fabrication.

Our laboratory has developed polyurethane materials based on lysine and glycerol that degrade hydrolytically into non-toxic components – chiefly lysine, glycerol and CO_2 [32,33]. These polymers can easily be fashioned into differing morphologies using standard urethane processing techniques. The degradation characteristics of these polyurethanes have proven useful in the design of various drug delivery systems [34–36]. The present study is an extension of previous work seeking to determine if the release of multiple chemotherapeutic agents at differing rates can be achieved from a single-phase polymer system. The delivery strategy is centered on the covalent incorporation of drugs into the polymer backbone, later to be released via passive hydrolysis of urethane and urea bonds. The chemical structures of DB-67 and doxorubicin contain different functional groups, and we propose to use them to form urethane and urea linkages with differing degrees of hydrolytic susceptibility (Table 1). Therefore, each drug should elute from the polyurethane material at a characteristic rate related to the functional groups present in the molecular structure of the chemotherapeutic.

2. Materials and methods

2.1. Materials

Lysine diisocyanate methyl ester (LDI) was purchased from Chemical Division, Kyowa Hakko Kogyo Ltd. (Tokyo, Japan). 7-*Tert*-butyldimethylsilyl-10-hydroxycamptothecin (DB-67) was obtained from Dr. Dennis Curran (University of Pittsburgh, Chemistry Department). All other chemicals were obtained from Sigma–Aldrich Chemical (Milwaukee, WI) and were of reagent grade unless otherwise specified.

2.2. Synthesis of drug-loaded polyurethane foams

Glycerol (mol. wt. 92.01, 0.600 g, 6.5 mmol), LDI (mol. wt. 212.20, 2.00 g, 9.5 mmol), 1,4-diazobicyclo[2.2.2]-octane (DABCO) (mol. wt. 112.18, 3.0 mg, 0.027 mmol) and anhydrous dimethyl sulfoxide (DMSO) (1.25 ml) were added to a small, dry 20 ml reaction flask; three different samples were prepared. Doxorubicin (mol. wt. 579.9, 5.0 mg, 8.6×10^{-3} mmol), and DB-67 (mol. wt. 478.7, 5.0 mg, 1.0×10^{-2} mmol) were each added to a different flask. To the third flask, the same amounts of doxorubicin and DB-67 were added. The flasks were flushed with nitrogen prior to being sealed to remove ambient moisture. The reactions were allowed to stir atop a magnetic stir plate in the dark at room temperature until the viscosity measured greater than 3.0×10^4 cP so that the foams would not collapse. At that time, the viscous pre-polymer was transferred to a 100 mm polytetrafluoroethylene dish and 100 μl of distilled, deionized H_2O (ddH_2O) was added. The samples were thoroughly mixed three times at 2 min intervals to ensure the ddH_2O was fully incorporated. The rising foams were left open to the atmosphere yet covered to protect each sample from light while curing. The resulting polymer foams were placed into a vacuum oven overnight at 25 °C. The foams were then placed into storage and protected from light at 4 °C for further analysis.

2.3. Drug reactivity with LDI

Solutions of doxorubicin and DB-67 were prepared in anhydrous DMSO at concentrations of 30.0 and 10.0 mg ml^{-1}, respectively. Two small (5 ml) reaction flasks were dried thoroughly and flushed with nitrogen, and DABCO (1.5 mg) was added to each flask. To one flask, 400 μl each of the DB-67 (0.025 mmol) solution and LDI (mol. wt. 212, 5.3 mg, 0.025 mmol) were added to give a 1:1 molar ratio of DB-67 to LDI. To the other flask, 200 μl each of the doxorubicin (0.003 mmol) solution and LDI (mol. wt. 212, 2.2 mg, 0.009 mmol) were added to give a 1:3 molar ratio of doxorubicin to LDI. In each case, a 1:1 isocyanate to total amine/hydroxyl ratio was obtained. Infrared spectra were recorded immediately upon addition of the LDI and 4 h later to determine the reactivity of each drug.

2.4. Excitation–emission fluorescent spectra and photostability

Stock solutions of DB-67 and doxorubicin were prepared in DMSO at a concentration of 1.0 mg ml^{-1}. Each sample was sonicated for 5 min at 25 °C to ensure complete

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