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# Catalyst-dependent drug loading of LDI–glycerol polyurethane foams leads to differing controlled release profiles

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**Abstract**

The purpose of the present study was to develop biodegradable and biocompatible polyurethane foams based on lysine diisocyanate (LDI) and glycerol to be used as drug-delivery systems for the controlled release of 7-*tert*-butyldimethylsilyl-10-hydroxy-camptothecin (DB-67). The impact of urethane catalysts on cellular proliferation was assessed in an attempt to enhance the biocompatibility of our polyurethane materials. DB-67, a potent camptothecin analog, was then incorporated into LDI–glycerol polyurethane foams with two different amine urethane catalysts: 1,4-diazobicyclo[2.2.2]-octane (DABCO) and 4,4′-(oxydi-2,1-ethane-diyl)bismorpholine (DMDEE). The material morphologies of the polyurethane foams were analyzed via scanning electron microscopy, and DB-67 distribution was assessed by way of fluorescence microscopy. Both foam morphology and drug distribution were found to correlate to the amine catalyst used. Hydrolytic release rates of DB-67 from the polyurethane foams were catalyst dependent and also demonstrated greater drug loads being released at higher temperatures. The foams were capable of delivering therapeutic concentrations of DB-67 in vitro over an 11 week test period. Cellular proliferation assays demonstrate that empty LDI–glycerol foams did not significantly alter the growth of malignant human glioma cell lines ($P < 0.05$). DB-67 loaded LDI–glycerol polyurethane foams were found to inhibit cellular proliferation by at least 75% in all the malignant glioma cell lines tested ($P < 1.0 \times 10^{-8}$). These results clearly demonstrate the long-term, catalyst-dependent release of DB-67 from LDI–glycerol polyurethane foams, indicating their potential for use in implantable drug-delivery devices.


*Keywords:* Lysine diisocyanate; Polyurethane; Drug-delivery; Camptothecin; Glioma

## 1. Introduction

Camptothecin (CPT) and its numerous synthetic analogs comprise a special class of anticancer agents that appear to be quite active in human lung, ovarian, breast, pancreas and gastric cancers previously reported resistant to chemotherapy [1–4]. CPT is a naturally occurring alkaloid that was first isolated from the Chinese tree *Camptotheca acuminata* (Nyssaceae) by Wall and coworkers [5]. It inhibits the enzyme topoisomerase I (topo I), a nuclear protein essential for DNA repair during replication [6,7]. Currently, two CPT analogs, topotecan and irinotecan, have been approved by the Food and Drug Administration (FDA), and at least 10 additional CPT derivatives are in various stages of clinical trials – including 9-amino-CPT

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in advanced clinical trials [3,4]. The CPT analog 7-*tert*-butyldimethylsilyl-10-hydroxy-camptothecin (DB-67) was synthesized in an attempt to enhance the stability and performance of CPTs [8–10]. DB-67 displays superior human blood stability relative to other camptothecin agents and possess a very high intrinsic potency against the topo I target enzyme. However, the compound is largely water-insoluble which has made clinical use quite difficult [11]. DB-67 has yet to be implemented in any clinical therapies due to an inability to be delivered in adequate quantities to impact tumor growth and disease progression.

Polyurethanes are a special class of synthetic materials that are widely used in many modern industrial applications as insulations, sealants, coatings and foams [12]. Many have gained FDA approval and currently find a place in numerous medical technologies due to their unique chemical and physical properties [13–17]. Polyurethanes are easily synthesized from diisocyanate and polyalcohol precursors via a condensation reaction that can be controlled by various urethane formation catalysts. The catalytic mechanisms and their effects on the resultant polymer architecture are well understood and can be tailored to specific material applications [18,19].

For the most part polyurethane catalysts can be classified into two distinct categories: organometallic and amine [20,21]. Furthermore, the reactions they catalyze can be broken down into three categories: blowing, gelling and cross-linking. The blowing reaction involves the reaction of isocyanate and water and generates a carbamic acid intermediate. As carbamic acid readily decomposes into an amine and $CO_2$, the polyurethane expands into foam. The gelling reactions simply refer to urethane or urea formation. The cross-linking reactions are generally quite limited and refer to the reaction of isocyanate with itself, urethanes or ureas. Organometallic catalysts are often tin-based and tend to be more selective for gelling reactions, catalyzing urethane and urea formation. Amine catalysts are chiefly tertiary amines and they catalyze both gelling and blowing reactions. In this study, we focus primarily on the tertiary amine catalysts 1,4-diazobicyclo[2.2.2]-octane (DABCO) and 4,4′-(oxydi-2,1-ethane-diyl)bismorpholine, also known as dimorpholino-diethyl ether (DMDEE). These two catalysts were chosen because DABCO is non-selective in nature and known to catalyze both gelling and blowing reactions, while DMDEE remains specific for the blowing reaction.

The stability and degradation characteristics of polyurethane materials are ideal for the construction of controlled release systems. However, the use of polyurethanes in the field of drug-delivery remains largely unexplored, although studies do exist that support their use [22–25]. Our laboratory has developed a new generation of biocompatible, biodegradable polyurethane constructed from lysine diisocyanate (LDI) and glycerol that degrade predictably via a hydrolytic mechanism into non-toxic components – lysine, glycerol and $CO_2$ [26,27]. These materials possess the same versatility as widely used commercial polyurethanes, easily being processed into forms with unique physical properties. We have previously synthesized LDI–glycerol films and demonstrated their use as long-term drug-delivery reservoirs [28]. However, we can utilize unique features of polyurethane chemistry to fashion these materials into foams possessing different drug-delivery characteristics. In this study, we have attempted to use the catalytic mechanisms of two different amine urethane catalysts to regulate the incorporation of drug and impact the overall release characteristics of our drug-delivery materials. The purpose of this study is to formulate and characterize a drug-delivery system based on hydrolyzable polyurethane foams prepared from LDI and glycerol.

## 2. Materials and methods

### 2.1. Materials

Lysine diisocyanate methyl ester (LDI) was purchased from Chemical Division, Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). 7-*tert*-Butyldimethylsilyl-10-hydroxycamptothecin (DB-67) was obtained from Dr. Dennis Curran (University of Pittsburgh, Chemistry Department). The human malignant glioma cell lines U87 and T98G were obtained from the American Type Tissue Culture Collection. The LN229 glioma cell line was kindly provided by Dr. Nicolas de Tribolet (University of Lausanne, Switzerland). The SG388 glioma cell line was provided by Dr. Suzanne Gollin (University of Pittsburgh, School of Public Health) and was established at the Children's Hospital of Pittsburgh from a pediatric malignant glioma specimen. MTS reagents were obtained from Promega (Madison, WI), and bismuth 2-ethylhexanoate was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals were obtained from Sigma–Aldrich (Milwaukee, WI) and were of reagent grade unless otherwise specified.

### 2.2. Urethane catalyst toxicity

T98G cells were plated in Dulbecco's MEM media at a density of 100 cells/well in 96-well plates. The plates were incubated for 24 h to allow for cell adhesion, and the media was changed prior to any treatment. Solutions of bismuth 2-ethylhexanoate (MW 639), tin(II) 2-ethylhexanoate (MW 405), dibutyltin dilaurate (MW 632), DABCO (MW 112) and DMDEE (MW 244) were prepared in dimethyl sulfoxide (DMSO); the final concentration of each solution was 10 mM. The catalyst solutions were mixed thoroughly, and 1.0 ml of each was diluted with 4.0 ml of Dulbecco's Minimum Essential Medium (MEM) to arrive at 2 mM solutions. Serial dilutions of the catalyst/media solutions were performed across two 96-well plates, halving the concentration with each dilution. After a 5 day incubation period, the number of viable T98G cells were determined by measuring the bioreduction of the tetrazolinium compound MTS by intracellular hydrolases in the presence of the electron coupling reagent PMS as previously described [29].

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