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RESEARCH ARTICLE

Evaluation of a perforated drug delivery system in mice for prolonged and constant release of a hydrophilic drug

Ashish Rastogi · Phillip D. Bowman · Salomon Stavchansky

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Abstract A drug delivery system (DDS) consisting of a perforated microtube (polyimide, inside diameter=1.8 mm, tube length=20 mm, hole size=0.15 mm) was characterized in vitro and in vivo for its usefulness for long-term release of hydrophilic drugs at a constant rate. Sodium fluorescein mixed with stearic acid was used as the model drug. The DDS was packed with sodium fluorescein and stearic acid in ratios of 50:50, 40:60, and 25:75, respectively, and in vitro drug release studies were performed in saline. Linear release rates with R^2>0.9700 were obtained for all groups. Release rates of 1,077.3±264.6, 342.6±146.4, and 14.4±7.0 μg/day for sodium fluorescein were obtained from the three groups, respectively. After monitoring the in vitro release of fluorescein for 11 days, 7 tubes from the 40:60 group were implanted subcutaneously in each individual mice to study the in vivo release of fluorescein from the tubes by measuring the fluorescein in the urine for 84 days. An initial rapid release during the first 4 days was followed by a near zero order fluorescence from the tubes (R^2=0.9870). Following completion of the study, the DDSs were retrieved for histology. Morphological analysis indicated no clinical adverse reaction at the site of device implantation specific to the device. The DDS was found to be biocompatible and capable of long-term constant release of a hydrophilic drug such as sodium fluorescein.

Keywords Drug delivery system · Microholes · Stearic acid · Sodium fluorescein · Controlled release

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or Department of Defense. This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulation, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

A. Rastogi · P. D. Bowman
US Army Institute of Surgical Research,
San Antonio, TX 78234, USA

A. Rastogi
e-mail: ashishr@mail.utexas.edu

A. Rastogi · S. Stavchansky (✉)
Division of Pharmaceutics, College of Pharmacy,
The University of Texas at Austin,
Austin, TX 78712, USA
e-mail: stavchansky@mail.utexas.edu

P. D. Bowman (✉)
US Army Institute of Surgical Research,
3650 Chambers Pass, Bldg. 3610,
Fort Sam Houston, TX 78234, USA
e-mail: phillip.bowman@us.army.mil

Introduction

Continuous drug therapy is often required for extended periods to treat different chronic diseased states [1–3]. Extensive research has been done to formulate drugs for extended release to ensure continuous release of therapeutic amounts to avoid frequent dosing, patient distress, and non-compliance [4–7]. Diffusion-controlled reservoir type implantable devices are one such approach to drug delivery systems (DDS) that can be used for systemic as well as local delivery of drugs [5, 6]. We have earlier demonstrated the efficacy of one such diffusion controlled perforated DDS to deliver hydrophobic drugs at linear rates in vitro [6, 8].

Polymeric microspheres that are used for encapsulating a variety of drugs are another popular controlled delivery vehicle. They are biocompatible, have high bioavailability, and can be used for long-term delivery of drugs [9, 10]. However, a major challenge of the microsphere based drug delivery is maintaining drug stability in addition to the poor control of drug release rates [9]. In contrast to microspheres, a reservoir-type device, such as the perforated DDS described in this study, protects the drug somewhat, and is capable of long-term constant delivery of drugs.

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In this study, we have evaluated the perforated DDS in vivo and also tested its ability to deliver a hydrophilic drug. The primary objective of this study was to observe if the DDS was capable of delivering drugs at a linear rate for a long time (3 months) in vivo. Sodium fluorescein was used as a model drug because it is relatively non-toxic and readily detected in urine, which is easily collected non-invasively. A mixture of sodium fluorescein with stearic acid was loaded into the tubes. Lipids such as stearic acid and palmitic acid have been previously used to control the release of hydrophilic drugs [11–13]. In this study, stearic acid along with device parameters such as tube size, hole size, and hole number were used to control the release of sodium fluorescein from the perforated tubes.

Materials and methods

Materials

Polyimide tubing was obtained from Microlumen Inc. (Tampa, FL, USA). Sodium fluorescein and stearic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Heat shrink medical grade (USP Class VI) polyolefin tubing was obtained from Altex (San Antonio, TX, USA). Glass vials (2.0 ml) were obtained from Agilent (Santa Clara, CA, USA). Titanium wires of different diameters were obtained from Small Parts (www.smallparts.com).

Fabrication of holes and drug loading

Polyimide tubing (inside diameter=1.8 mm) was cut to 20 mm in length. A 0.15-mm hole was made with a titanium wire at the center of the tube. Mixtures of sodium fluorescein and stearic acid were prepared in different ratios of 50:50, 40:60, and 25:75, respectively. Each mixture was grounded to a fine powder and mixed thoroughly using a mortar and pestle. The perforated tubes (Fig. 1) were packed aseptically with these mixtures and the ends of the tube sealed with heat shrink polyolefin tubing.

In vitro drug release studies

The method validation was performed by preparing standard curve of sodium fluorescein in 0.01 M phosphate-buffered saline (PBS; pH 7.4) in the concentration range of 2–4,000 ng/ml. Linear regression analysis resulted in linear responses with the following regression equation: $y=3.0004x+90.034$ ($R^2=0.9992$). Intra-day and inter-day reproducibility was studied by generating standard curves three times a day for 3 days.

For in vitro drug release studies, the three groups of loaded tubes (50:50, 40:60, and 25:75) were placed in glass vials containing 1.8 ml of PBS, pH 7.4. Non-perforated polyimide tubes loaded with sodium fluorescein were used as controls in the study. The vials were placed on a rocker (46–48 oscillations/min) and maintained inside an incubator (37.0±1.0°C). At the time of fluorescein determination, the dissolution medium was replenished with fresh PBS and the fluorescein content in the withdrawn bulk fluid measured fluorometrically at emission and excitation wavelengths of 485 and 530 nm, respectively. The tubes were handled and maintained in an aseptic environment. After completion of the in vitro studies, the tubes with the closest release rates from the 40:60 group were reused for in vivo studies.

In vivo drug release studies

All in vivo studies were conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulation and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals and the US Army Institute of Surgical Research guidelines. Seven inbred FVB/NJ mice (Jackson Labs, Sacramento, CA, USA) aged 6 to 8 weeks were used in the study. The mice were anesthetized with 5% isoflurane–air mixture, and the dorsal surface of the mice was shaved. The drug-loaded tubes selected from the 40:60 in vitro group were implanted subcutaneously.

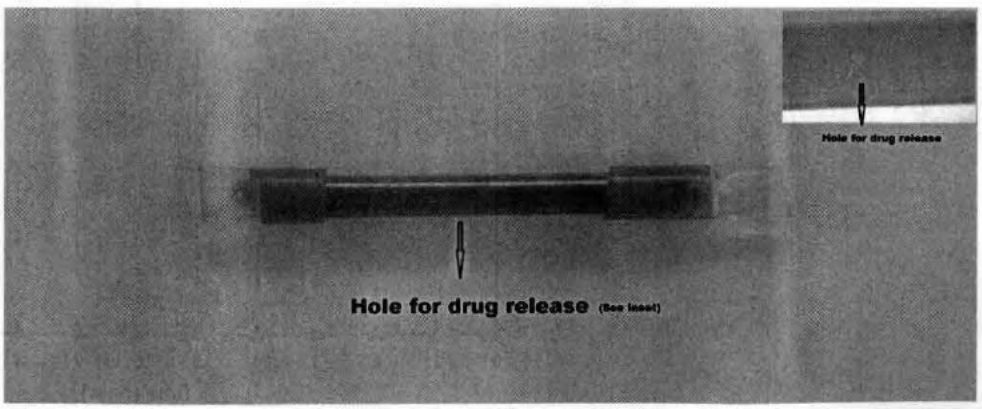

Fig. 1 Perforated polyimide delivery system with one microhole. The hole diameter, tube diameter, and tube length are 0.15, 1.8, and 20 mm, respectively. Loading capacity and release rate of the device can be controlled by the interplay of these parameters

Each mouse was housed individually in a metabolic cage (Tecniplast, Italy). The mice were kept under stable environmental conditions (12-h day–night cycle, 22±2°C) with free access to water and food. At each time point, a 24-h urine sample was collected from the collecting tube at the bottom of the metabolic cage between 0700 and 0900 hours. Pooled mouse urine collected prior to the device implantation served as blank. Urine samples were centrifuged, diluted using PBS, and analyzed fluorometrically at emission and excitation wavelengths of 485 and 530 nm, respectively. Cages were cleaned daily to avoid contamination from the previous collection and the samples were stored at −20°C.

Drug extraction and histopathologic analysis

Mice were euthanized and implanted tubes with associated tissues were retrieved from the back of each mouse and placed in 10% formalin overnight. The ends of the tubes were carefully removed and the remaining drug was extracted and the amount of fluorescein remaining was determined by fluorometry. After removal of the remaining drug, the DDS and associated tissue were prepared for histopathological analysis as described by Jaffe et al. [14] Briefly, the specimens were processed into paraffin and 5 μm sections were stained with hematoxylin and eosin and examined by light microscopy.

Statistical analysis

Linear regression analysis was performed on the cumulative release data and one-way ANOVA along with F statistics was used to estimate the association between the amount of release and time points. A difference of $P<0.05$ was considered significant.

Results

Hole size and drug loading

The diameter of the drilled holes was measured at 0.15± 0.05 mm (Fig. 1 inset). Mixtures of sodium fluorescein and stearic acid were prepared in different ratios of 50:50, 40:60, and 25:75, respectively. An average amount of 34.6±1.6 mg of the mixture was loaded in the tubes (1.76±0.08 mg/mm), independent of the mixture content. For the 40:60 mixture, this corresponded to approximately 14 mg of the sodium fluorescein loaded.

In vitro drug release studies

The release rates of sodium fluorescein/stearic acid mixture loaded tubes were evaluated in vitro over 11 days (Fig. 2). The mean release rate of 1077.3±264.6, 342.6±146.4, and 14.4± 7.0 μg/day for sodium fluorescein was obtained from the 50:50, 40:60, and 25:75 groups, respectively. The release rates were linear with R^2 values of 0.9907, 0.9921, and 0.9749, respectively. The linearity of release was further confirmed by F test, $F(1, 22)$, $P<0.05$.

In vivo drug release studies

The average fluorescence in urine was measured for a total of 12 weeks. The volume of the urine collected from the

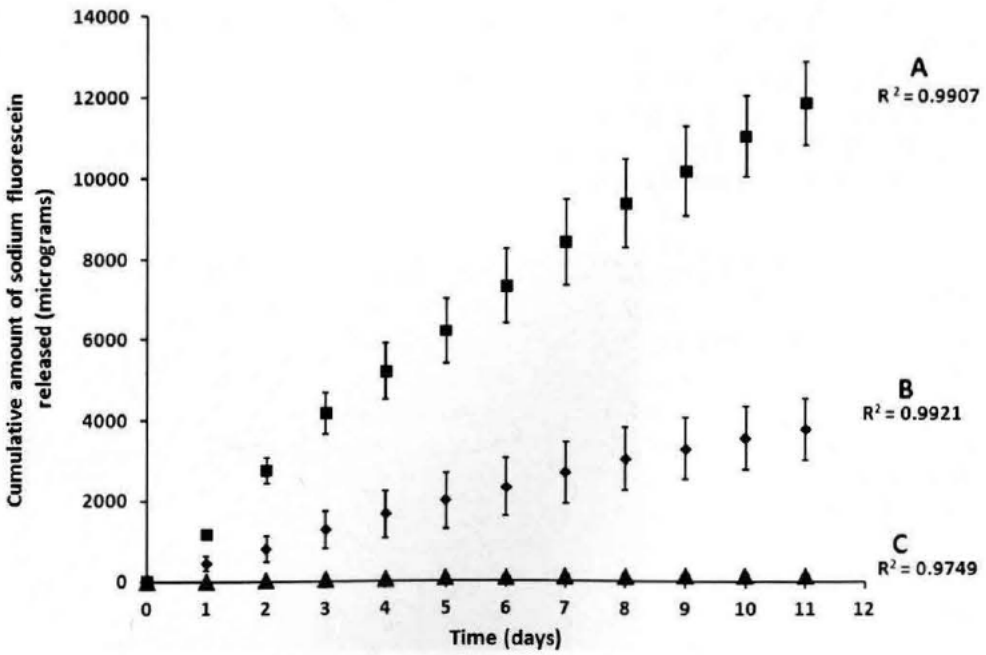

Fig. 2 Linear in vitro release rates obtained from different fluorescein: stearic acid ratios (n, R^2). **a** 50:50 (3, 0.9907), **b** 40:60 (11, 0.9921), and **c** 25:75 (5, 0.9740). The *bars* represent the standard deviations at each time point

metabolic cages was measured and was used to estimate the total fluorescence of the urine. The cumulative amount of the fluorescence observed in the urine, for the first 28 days, is illustrated in Fig. 3. The drug release from days 4 to 28 was described using the zero order rate equation, $C=K\,t$ (where, C is concentration, K is the zero order rate constant, and t is the time) [15]. During that period, the linear regression analysis of the cumulative data also indicates a near zero order kinetics with an R^2 value of 0.9870. The linearity of release rate was further confirmed by F test, F (1, 46), $P<0.05$. Rate excretion method was also used to analyze the results and the average amount of fluorescence observed in the urine per day was plotted against time-midpoint (T_{mid}; Fig. 4). A faster release with relatively high standard deviations was observed initially for the first 4 days. However, from day 4 onwards a constant of amount of fluorescence was detected in the urine for the duration of the study.

An average amount of 9.2±0.9 mg of fluorescein was recovered from the tubes at the end of the study. As 14 mg of fluorescein was initially loaded in the tubes, 65% of the drug was still inside the tubes after the in vitro and in vivo analysis. If the kinetics of the in vivo release do not change then the amount remaining in the tubes suggest that the drug release could have extended to several more months.

Histopathological analysis

Tubes implanted subcutaneously are surrounded by a thin layer of fibroblasts and collagen. There was little inflammatory reaction to the tube material that would be consistent with a minimally reactive foreign body. Inflammatory cells consist of rare macrophages and lymphocytes admixed with some cellular debris (Fig. 5).

Discussion

We have earlier demonstrated the feasibility of the DDS to deliver hydrophobic drugs in vitro [6]. This study was initiated as a proof of concept and is the first attempt to characterize the microperforated DDS in vivo. The DDS was also tested for its capability to continuously deliver hydrophilic drugs like sodium fluorescein for extended periods at a linear rate. Tube parameters such as drug solubility, tube size, and hole size can be modified to alter the release rate. The holes on the surface of the polyimide tube can be fabricated using different methods such as laser drilling or photolithography and have been previously described [6, 8]. In this study, the holes were manufactured manually using titanium wire. In our experience, holes greater than 0.15 mm can be easily manufactured using a cylindrical wire. We found that the 0.15-mm hole size was not suitable to achieve long-term release of a hydrophilic drug such as sodium fluorescein, as the release rate was very rapid. A simpler alternate was to use stearic acid to impart hydrophobicity to the compositions and to prolong the drug release by limiting the interaction of the hydrophilic drug with the dissolution medium. Stearic acid is also bioacceptable and biodegradable and hence a good candidate for drug delivery formulations. In addition, histopathological analysis did not reveal any clinically relevant inflammatory activity at the implanted site indicating the suitability of use of stearic acid/sodium fluorescein and polyimide/polyolefin as components of the DDS.

In this study, the same tubes were used in vitro and in vivo studies. The seven tubes from the former that had closest release rates were used with animals to decrease the inter-tube variability. In vitro and in vivo drug release plots were found to be in close relation as both had linear release kinetics with $R^2>0.9800$. We opted for measuring the amount of fluorescein in urine instead of that in plasma because the method is non-invasive and virtually pain free

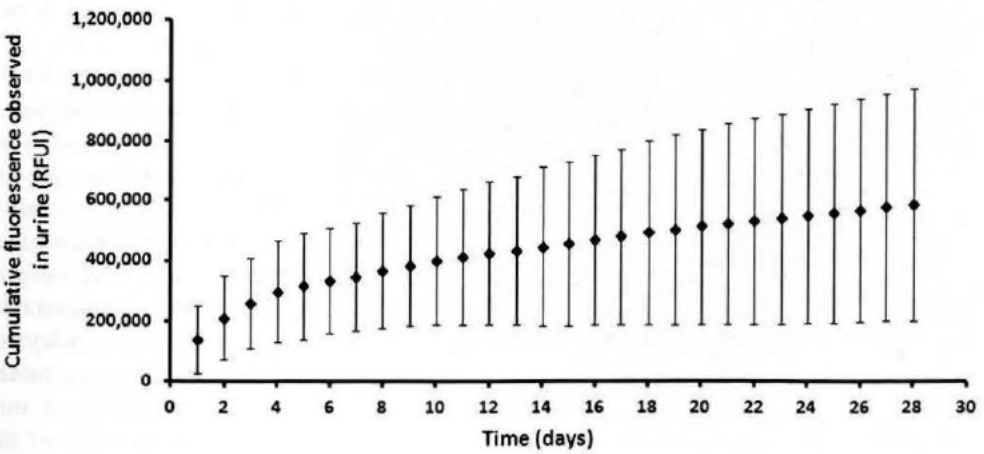

Fig. 3 Cumulative appearance of fluorescence in urine after implantation of the perforated polyimide DDS containing a 40:60 mixture of fluorescein/stearic acid. *Each point* represents the average ($n=7$) and the standard deviation

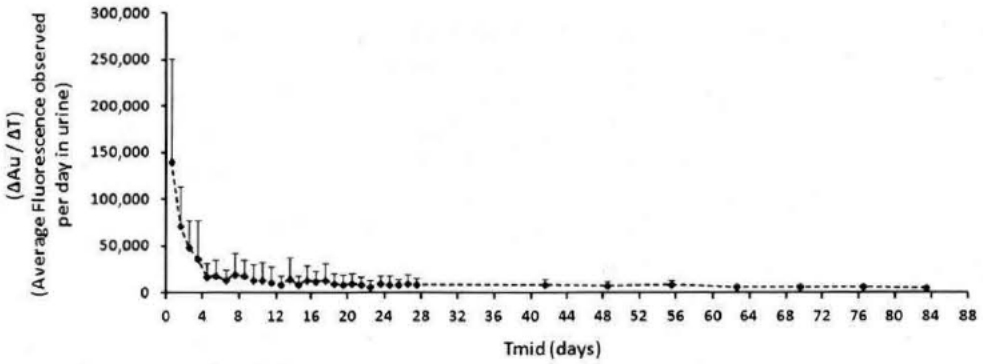

Fig. 4 The average rate of appearance of fluorescence ($\Delta Au/\Delta T$) versus the time-midpoint (T_{mid}) plot of the urine collection interval. The data indicate that a constant release rate is achieved after 4 days of equilibration of the delivery device with physiological conditions. The data are represented as mean with standard deviation ($n=7$)

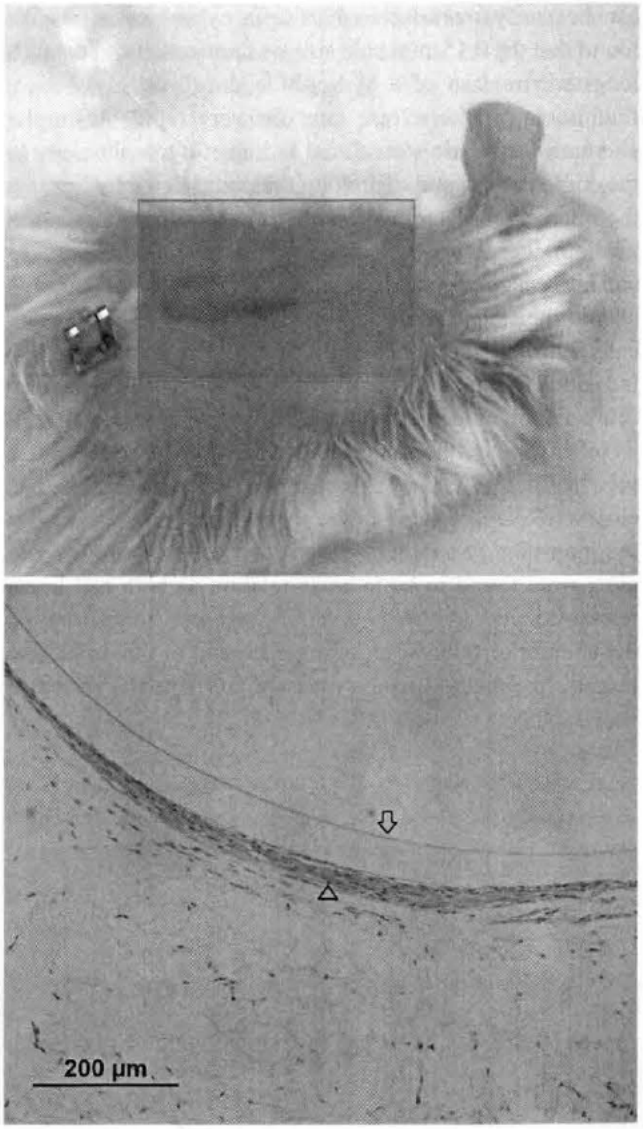

Fig. 5 (*I*) Subcutaneously implanted tube at 3 months before its retrieval. (*II*) Fibroblasts, collagen, and rare inflammatory cells (*arrow head*) were observed surrounding the polyimide tube (*arrow*)

for the animal. Urine data were first analyzed by plotting cumulative fluorescence against time (Fig. 3). The cumulative plot was useful to establish the linearity of the plot by calculating the R^2 value. However, after 28 days the urine samples were collected at different time points. As such, the cumulative data were only plotted for the first 28 days. To plot the entire urine data, rate of excretion versus time-midpoint plot was used (Fig.4). A significant advantage of the rate of excretion plot is that each data point is independent and a missed sample or variation in time interval between different data points is not critical. Additionally, since urine is collected over a discrete time interval, each time point is represented by the mid-point of the interval, T_{mid}.

When administered systematically, sodium fluorescein is metabolized to its conjugate, fluorescein monoglucouronide. It is widely distributed in the body tissues, conjugated by liver, and excreted by the kidney. In this study, we have reported the total fluorescence that was observed in the urine, which is a combination of both conjugated and unconjugated fluorescein. In order to estimate the amount of fluorescein excreted in urine per day, it is essential to convert the conjugated to unconjugated as well extract the drug from different tissues of the animal. However, the conversion and extraction steps seemed unnecessary for a model drug fluorescein as the primary objective of the study was only to investigate if the DDS is capable of a long-term and zero order release in vivo. Hence, the fluorescence values are reported in RFU in the in vivo studies and not converted to micrograms. The plotting of fluorescence values in Figs. 3 and 4 suggest that the rate of release of fluorescein from the DDS is linear after day 4. As 65% of the drug was still remaining inside the tubes, a long-term drug delivery comprising of several months can be expected at the present release rate.

A possible explanation for the rapid initial release may relate to the injury to the tissue during the subcutaneous implantation resulting in movement of interstitial fluid from blood into the area [16]. The initial increased movement of interstitial fluid initially drives

a greater amount of fluorescein out of the device. However, after several days, the microvessels are restored to the preinjury level and the rate of removal slows. Growth of cells over the hole by day 3 or 4 may also contribute to the ultimate reduction in drug delivery. The data presented suggest that the equilibration of the device with the physiological environment takes about 4 days as reflected by the stabilization of the release rate (Fig. 4).

As approximately 65% of the sodium fluorescein could be extracted out of the DDS after retrieval from the mice, it indicated that the DDS was capable of delivering hydrophilic drugs for prolonged periods at near zero order rates. It may be possible to extend the application of the DDS for local or systemic delivery of potent drugs, proteins, and peptide-based drugs that have become important therapeutic tools to manage chronic diseases [17]. Greater use of these proteins and peptides is limited by poor oral bioavailability, short half-life, and degradation in the stomach and intestine, and poor adsorption due to size or polar/charge distribution [18, 19]. High molecular weight also limit their delivery by transdermal route and presence of enzymes in eye tissues and nasal mucosa make ocular and nasal route unfavorable as well [20]. It may also be possible to use the DDS for delivery of such labile drugs via the subcutaneous route as the device will act as a protective carrier limiting the interaction of the drug to the hostile physiological environment.

Furthermore, the DDS may be used to deliver drugs such as anti-inflammatory agent, antihypertensive agent, antipsychotic agent, antidepressant, anti-diabetic agent, an antitumor agent, or combinations thereof that are required for long-term management of chronic diseases. However, a thorough analysis of any active agent in combination with the DDS in an animal model is necessary to ensure that sustained therapeutic levels are maintained and unwanted effects are avoided.

Conclusion

The DDS has been earlier characterized, in vitro, for its capability to release hydrophobic drugs in a linear manner over an extended period. The present investigation was the first study to evaluate the perforated DDS in vivo. The results of this study suggest that in combination with lipids such as stearic acid, it may be feasible to use the DDS for long-term and zero order release of hydrophilic drugs, such as sodium fluorescein. The concentration gradient across the hole is the main driving force for release of drug from the perforated device.

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