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Featured Article

Dosage Form Development, *in Vitro* Release Kinetics, and *in Vitro–in Vivo* Correlation for Leuprolide Released from an Implantable Multi-reservoir Array

James H. Prescott,[1,2] Timothy J. Krieger,[1] Sara Lipka,[1] and Mark A. Staples[1]

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Purpose. Implanted multi-reservoir arrays improve dosing control relative to osmotic pumps or polymer depots. The limited reservoir volume requires concentrated formulations. This report describes the development of a stable solid phase formulation of leuprolide acetate for chronic *in vivo* delivery from a multi-reservoir microchip and examines the correlation between *in vitro* release kinetics and serum pharmacokinetics.

Materials and Methods. Concentrated formulations (>10% w/v) were prepared using small volume processing methods. Drug yield, release kinetics, and formulation stability were evaluated *in vitro* by HPLC. The correlation between *in vitro* and *in vivo* kinetic data was determined for a solid formulation by direct comparison of data sets and using absorption kinetics calculated from the Wagner–Nelson equation.

Results. High yield and the control of release kinetics by altering peptide formulation or reservoir geometry were demonstrated. Lyophilized leuprolide in a soluble solid matrix exhibited reproducible release kinetics and was stable (>95% leuprolide monomer) after 6 months at 37°C. A strong correlation was found between *in vitro* release kinetics and *in vivo* absorption by direct comparison of data sets and using the Wagner–Nelson absorption (slopes of 1.01 and 0.91; R^2 0.99).

Conclusions. Reproducible releases of a stable solid leuprolide formulation from a multi-reservoir microchip were achieved *in vitro*. Chronic pulsatile release was subsequently performed *in vivo*. Comparison of *in vitro* and *in vivo* data reveals that pharmacokinetics were controlled by the rate of release from the device.

KEY WORDS: drug delivery; implant; *in vitro–in vivo* correlation; microchip; protein and peptide formulation.

INTRODUCTION

An increasing fraction of new pharmaceutical products are biological macromolecules (i.e., peptides, proteins, oligonucleotides, and genes) that exhibit the therapeutic advantages of high specificity, low toxicity and, in some cases, high potency. The most prevalent and preferred mode of drug administration, oral delivery, fails for many biological macromolecules because they exhibit poor oral bioavailability (1). Therapeutic biomolecules generally require parenteral administration (2). Alternatives to injection of therapeutic biomolecules include pulmonary ([ex.] EXUBERA® (insulin human [rDNA origin])) and implantable systems: polymer depots ([ex.] Gliadel® Wafer (polifeprosan 20 with carmustine implant)) and osmotic pumps ([ex.] Viadur™ (leuprolide acetate implant)).

Implantable drug delivery devices are particularly desirable when compliance with a prescribed drug regimen is critical or to avoid delivery by repeated injection. Currently available implantable delivery systems have limited dosing flexibility and may require solution phase formulations that are stability limiting for biological macromolecules. Macromolecular therapeutics frequently require chronic and nonlinear (pulsatile) administration that polymer depots and osmotic pumps cannot provide. Implantable pumps have been developed to deliver drugs in either a pulsatile or continuous manner. However, these devices are relatively large (3), are susceptible to clogging (4,5), and are limited to delivering solution phase drug formulations. The preferred drug delivery implant will be small in size, protect the drug from physiological fluids that can accelerate drug decomposition, control release kinetics and dose, and be controllable by physician or patient. An array of individually sealed, drug-containing reservoirs that can be opened on command to deliver a dose to the body meets these criteria.

This work describes the development of a concentrated formulation of leuprolide for delivery from an implantable multi-reservoir array. The array of discrete reservoirs provides

[1] MicroCHIPS, Inc., 6-B Preston Court, Bedford, Massachusetts 01730, USA.
[2] To whom correspondence should be addressed. (e-mail: jprescott@mchips.com)

ABBREVIATIONS: DMSO, dimethylsulfoxide; IVIVC, *in vitro–in vivo* correlation; PEG, polyethylene glycol; PK, pharmacokinetic; RSD, relative standard deviation; SD, standard deviation.

precise dosing control (including the option to terminate therapy without device removal) and the flexibility to use solution phase or solid phase formulations. Formulation stability and *in vitro* release properties are demonstrated. The luteinizing hormone-releasing hormone analog leuprolide acetate is the active pharmaceutical ingredient in a number of marketed products for the treatment of prostate cancer, endometriosis, and precocious puberty. Leuprolide was chosen as the model compound for this study because it is representative of therapeutic polypeptides with poor oral bioavailability, established analytical methods exist, and it is highly soluble and exhibits good stability in polar solvents (6–8). The reservoir array design, fabrication, and release activation mechanism have been described previously (9), and it has been shown that multi-reservoir arrays containing discrete doses of drug within a programmable implantable device can provide pulsatile on-demand delivery of leuprolide *in vivo* for 6 months (10).

The current studies describe the *in vitro* performance of the multi-reservoir device with a range of solution phase, solid phase, and semi-solid phase leuprolide formulations, leading to the development of the novel solid phase formulation that was tested *in vivo*. All formulations tested in this study were obtained from filling solutions having peptide contents in excess of 10% (w/v). Examples are provided of formulation, filling, lyophilization, and post-lyophilization processing within the small volume (< 300 nl) of the reservoirs. Results demonstrating the reproducibility of drug release kinetics from the multi-reservoir array, and how release kinetics can be altered by modifying either the formulation or the reservoir geometry, are provided. A strong correlation is found between *in vitro* release kinetics and previously reported pharmacokinetics for a stable formulation of lyophilized leuprolide in a solid, water soluble matrix.

MATERIALS AND METHODS

Materials

All chemicals were used as received without further purification: leuprolide acetate (PolyPeptide Laboratories, Torrance, CA), polyethylene glycol 300 (PEG 300) (300 Da, mp < 0°C, Sigma-Aldrich, St. Louis, MO), polyethylene glycol 1450 (PEG 1450) (1,450 Da, mp 42°C, Mallinckrodt Baker, Phillipsburg, NJ), dimethyl sulfoxide (DMSO) and 1,6-hexane diol (Alfa Aesar, Ward Hill, MA), pressed neutral phosphate buffer tablets (Calbiochem, San Diego, CA), sodium azide (Sigma-Aldrich), and Polysorbate 20 (Tween 20, Sigma-Aldrich). Microchip devices were fabricated as described previously (9) by Micralyne, Inc. (Edmonton, CANADA). Isotonic, neutral phosphate buffer was prepared by dissolution of pressed buffer tablets (PBS, 10 mM sodium phosphate, 140 mM NaCl, 3 mM KCl, pH 7.4) in water containing 0.05% sodium azide and 0.004% Tween 20, and was used as the flow cell mobile phase. Chromatographic analysis was performed with HPLC grade acetonitrile and water (Mallinckrodt Baker). Trifluoroacetic acid (TFA, Pierce Biotechnology, Rockland, IL) was used to modify the pH of the HPLC mobile phase. Reservoir filling apertures were sealed with an adhesive aluminum film (Axygen Scientific, Union City, CA).

Preparation of Leuprolide Solutions

Leuprolide acetate (85% peptide by weight, >98% peptide purity) solutions were prepared at small scale, typically less than 250 μl, by the direct addition of solvent to the solid leuprolide. The solvents were water and DMSO. Combining ratios of solvent to leuprolide were selected to yield peptide concentrations that were below the leuprolide solubility limits (7,8).

Chromatographic Analysis

Quantitative leuprolide concentrations were determined by reverse phase high performance liquid chromatography (RP-HPLC). The chromatography was performed on an Agilent Series 1100 HPLC (Agilent Technologies, Wilmington, DE), using a octadecylsilane stationary phase (Vydac; part number 218TP5215; 5 micron silica; 0.21 cm × 15 cm column). Gradient mobile phase conditions were employed (82% water and 18% acetonitrile (v/v) with 0.1% TFA from 0 to 3 min, linear gradient to 58% water and 42% acetonitrile with 0.1% TFA from 3 to 54 min), with a flow rate of 0.2 ml/min. Analyte detection was by absorbance at 215 nm. Concentrated leuprolide solutions required dilution prior to analysis. The maximum leuprolide concentrations in the *in vitro* release fractions typically exceeded the limit of quantitation by a factor of 100. The same method was used to evaluate leuprolide stability.

Reservoir Filling

The peptide concentration of the filling solutions ranged from 100 mg/ml to approximately 400 mg/ml. The multi-reservoir arrays used in this work were contained in silicon-glass (silica) composite microchips. Representative images of the microchip and of an individual reservoir are presented in Figs. 1 and 2 (10). Prior to the filling operations, microchips were mounted on an automated stage which provided precise positional control in the x–y plane, and the position of the syringe pump was precision controlled in the z-axis. The reservoir filling apparatus (Fig. 3) was assembled using commercially available hardware and a commercial software development system (LabVIEW, National Instruments Corp., Austin, TX), although the design and assembly of the hardware and software that control chip location, dispensing parameters, and other filling operation variables was performed in-house.

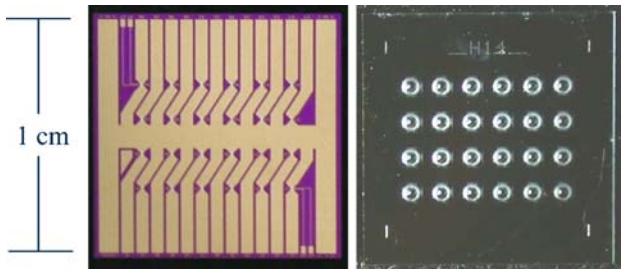

Fig. 1. Representative images of the microchip containing the multi-reservoir array that was used in this study (10). Front (*left*) and back (*right*) images of a 1 cm^2 microchip are shown. Reservoir filling is performed through the reservoir openings on the back of the chip. The chip is 1 mm thick, and the volume of each reservoir is approximately 290 nl.

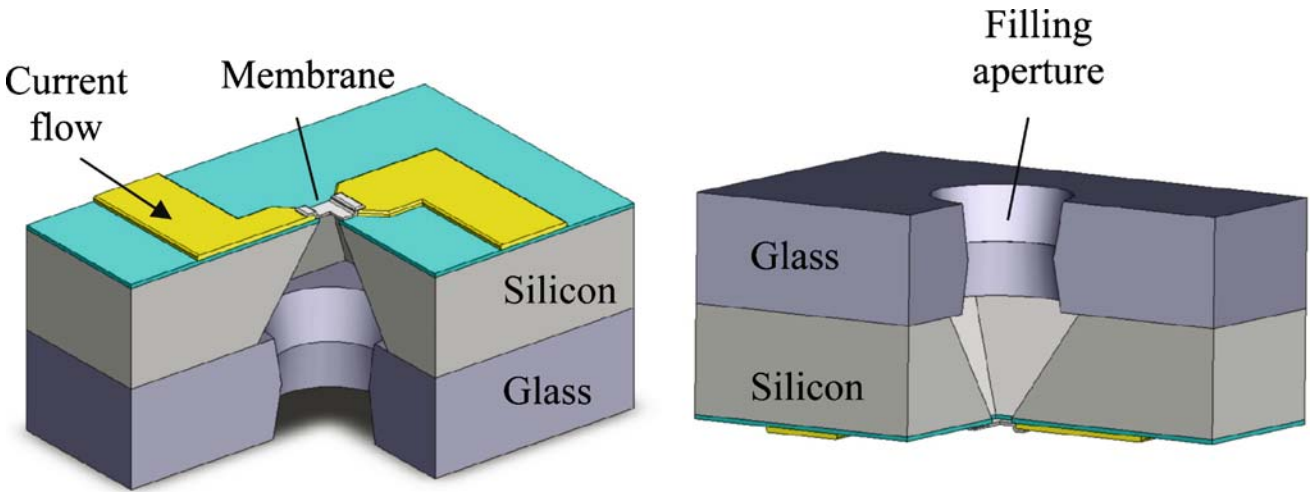

Fig. 2. Cross-sectional representations of a single reservoir of a multi-reservoir microchip (10). The conductive trace in series with the metal reservoir seal (membrane) is visible in the image on the *left*. The membrane is electrothermally removed within 100 ms with a pulse of current (~1 A) to initiate the release of the reservoir contents. The schematic has been inverted in the image on the *right* to provide a representation of an individual reservoir and of the aperture for filling the reservoir. (Not drawn to scale).

Leuprolide solution was dispensed into individual reservoirs through the openings on the back side of the microchip (Fig. 2). An automated microsyringe pump (World Precision Instruments, Sarasota, FL) delivered the solutions to reservoirs with high precision and accuracy (100 nl/dispense, 97% recovery with respect to the filling solution concentration, RSD 3.3%, $n = 21$). All liquid dispensing operations were performed using a 32-gauge needle (nominal ID 110 μm, nominal OD 220 μm). The syringe was mounted in a thermostatted block which maintained solid materials (i.e., PEG 1450) in a molten state during dispensing. Peptide solutions and PEG 300 were delivered at ambient temperature (<30°C). PEG 1450 and 1,6-hexanediol were delivered at temperatures exceeding their melting point (>42°C). As the filling operation began, the syringe needle entered a reservoir and then was withdrawn as dispensing occurred. Withdrawal of the needle while dispensing prevents the displacement volume of the needle from causing a fill overflow. After a reservoir was filled, the chip was automatically repositioned and the next reservoir(s) in the filling sequence was filled. Fill duration was approximately 2 s/reservoir, although faster rates could be achieved with the filling instrumentation. A thermostatted block on the movable stage (Fig. 3) permitted microchips to be held at temperatures slightly above the dew point to reduce the potential for evaporative losses while preventing moisture from condensing on the surface of the microchip. The filling station configuration of Fig. 3 permits the automated filling of up to six microchips per filling run, although the system could be modified to allow larger batches. Formulations that were not receiving further processing (i.e., select solution-phase fills) were sealed immediately after filling. For the purpose of performing *in vitro* releases, individual reservoirs were sealed with an adhesive aluminum film.

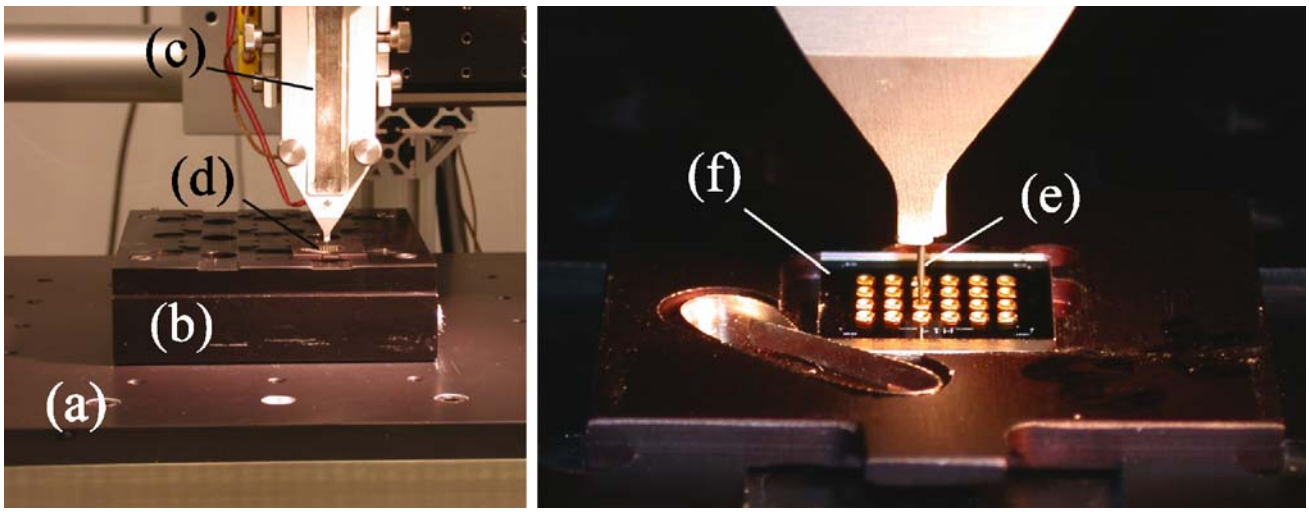

Fig. 3. The image on the *left* is of the automated microchip filling station, showing the movable stage (**a**), the thermostatted chip-holding block (**b**), syringe pump (**c**), and a single microchip (**d**). The image on the *right* is of a single reservoir being filled, showing the syringe needle (**e**) and the chip being filled (**f**).

Lyophilization and Processing within the Multi-reservoir Array

Immediately after the reservoir array in a chip to be lyophilized was filled with aqueous leuprolide solution, it was transferred to a pre-chilled shelf (−50°C) of a freeze-dryer (Advantage EL, VirTis, Inc., Gardiner, NY), and the reservoir contents were lyophilized using the non-optimized conservative cycle described by Table I. No efforts were made to minimize the length of the cycle in these studies, although the high surface area to volume ratio of the (frozen) solution in the reservoir likely facilitates lyophilization.

When the release of the reservoir contents is initiated by the removal of the membrane cap (Fig. 2), the formulation must come in contact with an external fluid for drug release to occur. The void volume within the reservoir and lyophilized cake may impede drug release if it forms a bubble within the reservoir at the opening. To compensate for this potential situation, the void volume within the lyophilized leuprolide cakes was displaced with water miscible materials in which leuprolide was poorly soluble. Several void volume displacing agents were considered, including polyethylene glycols with a range of molecular weights and melting points, and 1,6-hexane diol. The introduction of PEG 300 (mp < 0°C) produced a lyophilized solid in a fluid matrix. Two of the agents, PEG 1450 and 1,6-hexane diol (mp *ca.* 42°C in each case), were dispensed as a melt which filled the void space within the lyophilized cake and quickly solidified to yield a lyophilized solid in a solid matrix.

In Vitro Release Experiment

The filling apertures of filled chips were sealed with an adhesive aluminum film. Electrical connections to the conductive traces on the face of the microchip were established by wirebonding the traces to a printed circuit board/flex cable assembly (9). The chip assembly was mounted in a flow cell (Fig. 4), and ports on the opposite sides of the flow cell were connected to tubing through which fluid flowed unidirectionally across the microchip face. A schematic diagram of the *in vitro* release testing apparatus is presented in Fig. 5. This assembly permitted the controlled release activation of specific reservoirs and a controllable environment for studying release properties *in vitro*. The use of multiple parallel ports provides a plug-flow of mobile phase across the chip face. The inlet ports of the flow cell were connected to a reservoir containing neutral phosphate buffered saline solution (PBS). This flow cell mobile phase also contained 0.05% sodium azide and 0.004% Tween 20. The outlet ports were connected to an automated fraction collector. The entire system volume, including the volume over the face of the microchip, remained completely filled with buffer throughout each *in vitro* release experiment. Immediately before a release was initiated 4 ml of buffer was pumped through the cell (2 min at 2 ml/min), and the effluent fraction collected. The flow was then terminated ("stop-condition"), and the release of drug was initiated by electrothermal removal of the reservoir-sealing membrane. After 90 min another 4 ml volume of buffer was pumped through the flow cell ("flow-condition") and the effluent fraction collected. The volume of solution pumped through the cell was sufficient to completely flush the system. A new fraction was collected every 90 min for 24 h following release initiation, at which point leuprolide was no longer detectable. The quantitative leuprolide content of the fractions were determined by RP-HPLC, as described above.

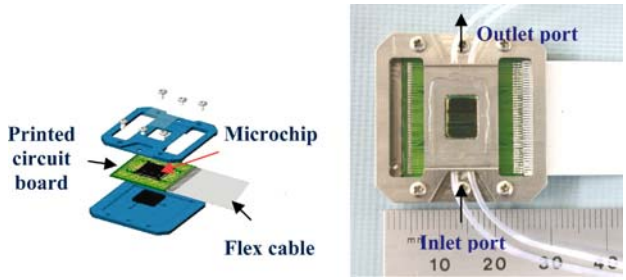

Fig. 4. Images of the flow-cell apparatus used for *in vitro* leuprolide release experiments. The flex cable, shown attached to the printed circuit board, delivers the release activation energy from a remote power supply.

Table I. On-chip Lyophilization Cycle[a]

Thermal Treatment			
Temperature (°C)	Time (min)	Pressure (mT)	Ramp (R) or Hold (H)
10	10	ambient	H
−50	60	ambient	R
−50	120	ambient	H
Drying phase			
−50	60	100	H
−50	900	30	H
5	55	30	R
5	120	30	H
25	20	30	R
25	180	30	H
20	10	500	R

[a] The condenser temperature was set to −60°C.
Reservoir arrays were not filled and placed in the lyophilizer until the shelf temperature reached −50°C.

Formulation Stability

The stability of two formulations was monitored for 6 months at 37°C. One formulation was a lyophilizate from a 24% (w/v) aqueous leuprolide solution, and the other was a lyophilizate from the same solution in a solid matrix of PEG 1450. The lyophilizates were obtained from 50 µl aliquots of aqueous leuprolide solution in glass vials using the lyophilization cycle of Table I. To produce the second formulation, lyophilized leuprolide in a matrix of solid PEG 1450, molten PEG 1450 (80°C) was introduced to the lyophilizate at ambient temperature. The volume of molten PEG 1450 dispensed per vial was 200 µl. Samples were maintained in sealed vials throughout the study. Air was not purged from the vials prior to sealing. Stability was assessed by dissolution of the samples in PBS and analysis by RP-HPLC. Three samples of each formulation were analyzed at each time point.

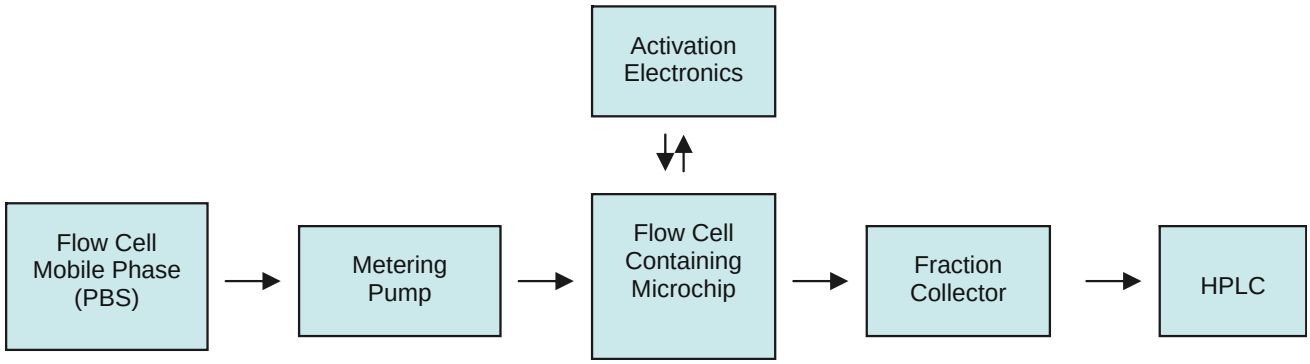

Fig. 5. Schematic representation of the *in vitro* release testing apparatus. A programmable HPLC pump was used as the metering pump.

In Vitro–In Vivo Correlation (IVIVC)

The chronic release of leuprolide from implanted multi-reservoir arrays has been described in detail previously (10). Briefly, devices containing the filled and sealed reservoir arrays were implanted in the subcutaneous tissue of male beagle dogs ($n = 6$). Individual reservoirs contained the solid formulation of lyophilized leuprolide in a matrix of PEG 1450, with 25 μg of leuprolide in each reservoir. One device was implanted per animal. Blood was drawn at intervals after each release activation. Serum was isolated from the whole blood samples, and serum leuprolide concentrations were determined by liquid chromatography—tandem mass spectrometry.

A study of the absorption, distribution, metabolism, and excretion (ADME) behavior for this leuprolide-release system combination was not performed. In the absence of absorption data the serum pharmacokinetic (PK) curve has been integrated, the area under the curve (AUC) normalized, and direct comparisons performed between the post-release mass recovery *in vitro* and the fractional AUC from the serum PK profile as a function of time post release activation following the example of Uppoor (11). The sampling time points were different for the *in vitro* and *in vivo* experiments. Therefore the *in vitro* and *in vivo* release kinetic profiles were interpolated at common time points for the 24 h following each release event, at which point leuprolide was no longer detectable. The IVIVC plot compares interpolated *in vitro* release and *in vivo* absorption fractions at the common time points. The PK data was also used to calculate fractional absorption by application of the Wagner–Nelson equation (Eq. 1) (12), and an IVIVC was determined for the *in vitro* release kinetics and the Wagner–Nelson absorption kinetics.

$$\begin{pmatrix} \text{fraction of dose} \\ \text{absorbed} \\ \text{at time t} \end{pmatrix} = \frac{C_{\text{serum},t} + k_{\text{el}}[\text{AUC}]_0^t}{k_{\text{el}}[\text{AUC}]_0^\infty} \qquad (1)$$

assuming $[\text{AUC}]_0^\infty = [\text{AUC}]_0^{24\text{hr}}$,

where $C_{\text{serum},t}$ is the measured serum leuprolide concentration at time t and k_{el} is the elimination rate constant

RESULTS AND DISCUSSION

Leuprolide Solution Preparation, Dispensing, and Lyophilization

Reservoir filling was performed with solution-phase peptide. In all cases for these studies, leuprolide solutions were formulated by adding solvent to peptide powder. Dissolution appeared complete in water and in DMSO, and minimal agitation was required to wet the powder and dissolve the peptide. Peptide concentrations in excess of 400 mg/ml could be achieved, although most work was performed at concentrations below 300 mg/ml. While the peptide solutions appeared more viscous than neat solvent, the solutions were readily dispensable into microchip reservoirs. Lyophilization of aqueous leuprolide solutions, performed within the reservoirs, yielded lyophilizates that displayed a low porosity surface. The lyophilizates often were cracked, which may be correlated to the high peptide concentration of the solutions. The appearance did not change during storage. These observations are consistent with prior experiences in our lab lyophilizing concentrated (>10% w/v) solutions of various therapeutic peptides within microchip reservoirs.

In Vitro Release Kinetics and Formulation Composition

A range of leuprolide formulation conditions and physical states were screened with the objective of identifying a formulation that was stable at 37°C for 6 months or longer, which provides reproducible release kinetics *in vitro*, and which could be used for *in vivo* studies. Aqueous solutions were considered as potential solution formulations and as a process step in producing lyophilized formulations.

The high porosity of the lyophilized product is a potential disadvantage for drug delivery applications. When liquid enters the reservoir after it is opened, the air within the matrix and any open space within the reservoir can form a bubble which can block the reservoir opening and prevent or retard dose wetting and dissolution, resulting in variable drug release behavior. As a safeguard to ensure release consistency *in vivo* any air pockets within the lyophilizate and reservoir were displaced. Post lyophilization, in-reservoir processing involved the addition of either PEG 300 (mp < 0°C), PEG 1450 (mp 42°C), or 1,6-hexanediol (mp 42°C) to the lyophilized cake. The resultant binary formulations were semi-solid at

Table II. Summary of the Times to 50% Leuprolide Recovery following Release Activations of Different Formulations

Formulation Description	Leuprolide Concentration in the Filling Solution (mg/ml)	Physical State at 37°C	Mean Time to 50% Recovery (min)	SD of Mean Time to 50% Recovery (min)	Replicates (n)
leuprolide in DMSO	169	solution	80	53	5
	416	solution	66	9	5
leuprolide in water	202	solution	105	22	3
	432	solution	132	NA	2
lyophilized leuprolide in PEG 300	194	solid/liquid	113	19	4
lyophilized leuprolide in 1,6-hexane diol	260	solid/solid	180	28	11
lyophilized leuprolide in PEG 1450	260	solid/solid	216	31	5
lyophilized leuprolide	252	solid	558	115	19

SD standard deviation; *NA* not applicable.

37°C in the case of PEG 300 and solid at 37°C in the cases of PEG 1450 and 1,6-hexanediol.

Formulation screening activities also considered solution phase formulations comprised of leuprolide in either water or DMSO, without further processing. The screening included the evaluation of *in vitro* release properties from multi-reservoir arrays. *In vitro* release testing was performed under the described standardized conditions. The fill volume for leuprolide solutions and for post lyophilization additions was 100 nl per reservoir. Release experiments were performed from reservoirs with a uniform geometry, having a nominal reservoir volume of 290 nl, and release opening of 2,500 μm^2 (aperture dimensions 50 $\mu m \times 50$ μm). A comparison of *in vitro* release kinetics, expressed as the mean time to achieve a 50% recovery of leuprolide following release activation, for a range of formulation compositions and physical states is provided in Table II.

The solution phase formulations clearly exhibit faster release kinetics than either solid–liquid or solid–solid formulations. The microchip device used in this work does not contain moving parts; after removal of the membrane cap, the drug release is governed by the rates of drug dissolution (solid and semi-solid forms) and diffusion (solution phase forms). To a first approximation, the mass recovery for a diffusion controlled (Fickian) release should be a linear function of the square root of time (13) (Eq. 2). Comparative release profiles for one of the solution phase formulations (leuprolide in DMSO at 416 mg/ml) and one of the solid formulations (lyophilized leuprolide in PEG 1450) are presented in Fig. 6. The release of the solution phase formulation is clearly faster, although the observed mass recovery of leuprolide is non-Fickian. The release kinetics of the solid phase formulation should be influenced by the rates of dissolution and diffusion. However, after an initial delay the observed release profile is nearly linear with the square root of time (i.e., Fickian in appearance for >75% of the recovery). While observed trends are predicable with respect to the relationship between the rate of release and the physical state of the formulation, additional factors that influence release kinetics (i.e., reservoir and dose shape and temporal changes in the dose shape) must account for the discrepancies between the theoretical and actual release rates for simple diffusion controlled release.

$$\text{Fickian Diffusion}: \begin{pmatrix} \text{mass} \\ \text{released or} \\ \text{recovered} \end{pmatrix} = kt^{1/2} \qquad (2)$$

where k is a proportionality constant

Lyophilized Leuprolide in a Solid PEG 1450 Matrix; Release Kinetics and Peptide Stability

Acceptance criteria for the formulation to be used *in vivo* included a demonstration of drug stability (leuprolide purity) for the planned conditions (37°C) and period of use (six months) *in vivo*, and acceptably reproducible release kinetics *in vitro*. Examples of the release kinetics for a series of tested formula-

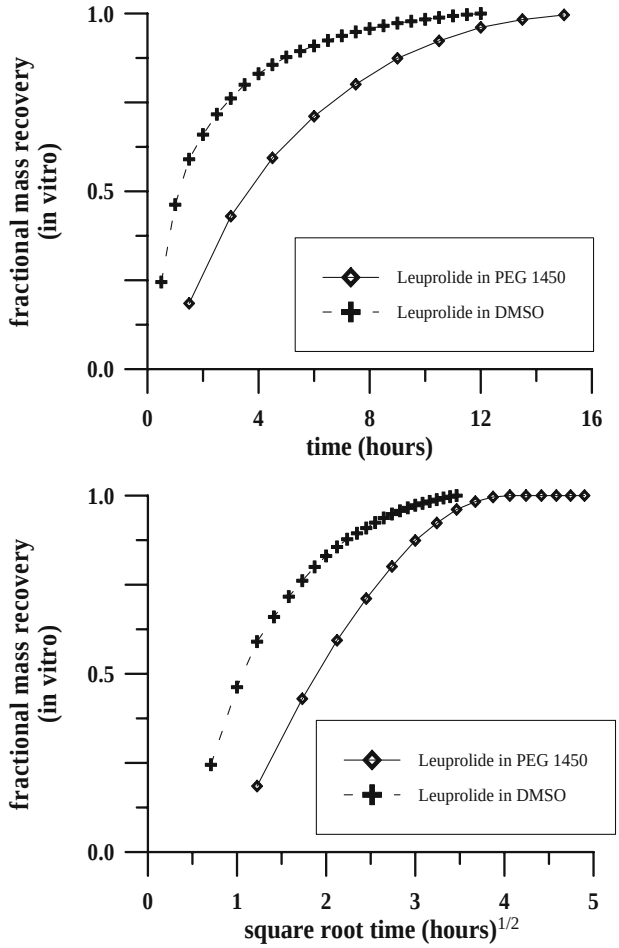

Fig. 6. Comparison between the *in vitro* release properties for a solution phase formulation of leuprolide in DMSO (416 mg/ml) and a solid phase formulation of leuprolide in PEG 1450 (26 micrograms leuprolide in 100 nl PEG 1450).

tions are presented in Table II. The formulation of lyophilized leuprolide in a matrix of PEG 1450 was ultimately selected for use *in vivo* due to its excellent stability profile, which is described below. The formulated filling solution was aqueous leuprolide acetate, leuprolide concentration 261 mg/ml, and the dispensed volume was 100 nl per reservoir. The volume of molten PEG 1450 introduced to displace the void volume was 100 nl per reservoir, and was sufficient to cover the lyophilized cake. The *in vitro* release results for a representative sampling of five consecutive releases from a multi-reservoir array are presented in Fig. 7. A single reservoir containing (nominally) 26 μg of leuprolide acetate was released for each release event. The area of the reservoir release aperture, open after the ablation of the sealing membrane, was 2,500 μm^2 (50 μm by 50 μm). This was the same aperture dimension used *in vivo*. Reproducible pulsatile release kinetics were obtained following release activation, with a mean time to 50% yield of 216 min (SD 31 min, $n=5$). The maximum leuprolide concentration was present in either the first or the second fraction collected during each release event. Leuprolide degradants were below the limit of detection (<1%) in the most concentrated fractions collected.

The formulation of lyophilized leuprolide in a solid matrix of PEG 1450 was placed on a stability study at 37°C.

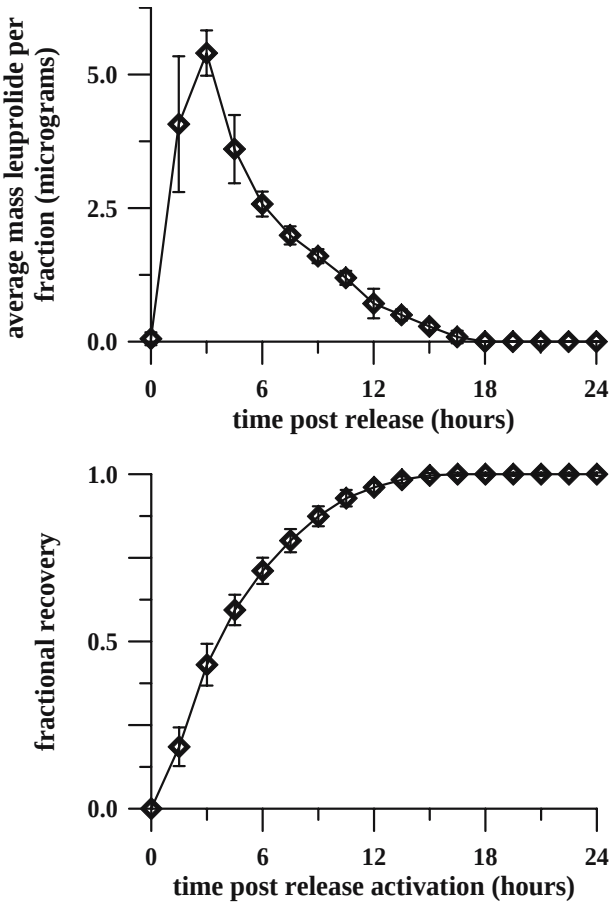

Fig. 7. Mean kinetic release profiles for five consecutive releases of lyophilized leuprolide in a PEG 1450 matrix. The membrane dimensions were 50 microns by 50 microns. The mean time to 50% recovery was 216 min (RSD 14%), and the coefficient of variation (CV) for the cumulative mass recovery was 1%. The *error bars* represent +/−1 SD of the mean.

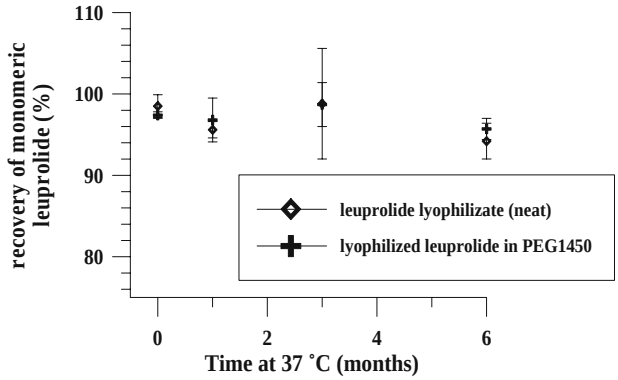

Fig. 8. Purity of lyophilized leuprolide acetate and of a formulation comprised of lyophilized leuprolide acetate with air displaced by PEG 1450. *Error bars* represent +/−1 standard deviation of the mean ($n=3$).

Lyophilized leuprolide without PEG 1450 was tested to control for the impact of PEG 1450 on leuprolide stability. The formulation of lyophilized leuprolide in a solid PEG 1450 matrix was stable for six months at 37°C (Fig. 8). RP-HPLC demonstrated 95.2% (SD 1.3%, $n=3$) monomeric leuprolide for lyophilized leuprolide in PEG 1450, and 93.9% (SD 2.2%, $n=3$) monomeric leuprolide for the lyophilizate after 6 months at 37°C. The physical appearance of both formulations was unchanged throughout the study. Other formulations that were screened, including lyophilized leuprolide in PEG 300, presented consistent visual appearance over six months. Because the formulation of lyophilized leuprolide in PEG 1450 was selected for *in vivo* work, these other combinations were not evaluated by HPLC.

The ability to prepare and store discrete doses in dry solid form within an implantable controlled release device is desirable. Degradation mechanisms that are often observed in solution phase, such as hydrolysis, aggregation, and redox chemistry, can be eliminated or significantly reduced by the removal of water and limiting molecular mobility during storage. Leuprolide exhibits good stability in aqueous solution at 37°C; the principle degradants are hydrolysis products, with approximately 90% recovery of monomeric leuprolide after 6 months (7). The current data indicate superior stability for

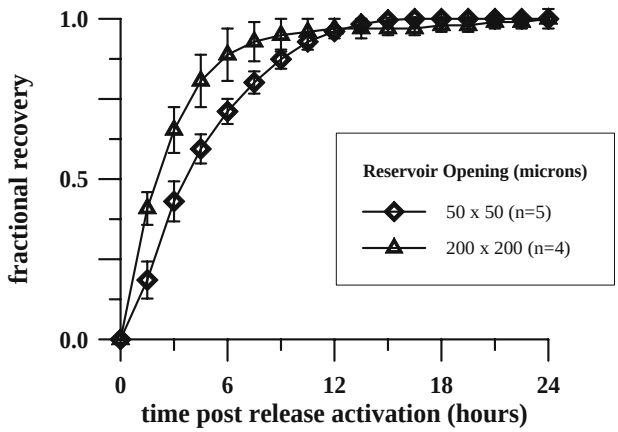

Fig. 9. Relationship between *in vitro* leuprolide release kinetics and the dimensions of the reservoir opening. The *error bars* represent +/−1 standard deviation of the mean.

Table III. Summary of Mass Recoveries and Recovery Times for a Formulation of Lyophilized Leuprolide in a PEG 1450 Matrix Released Through Reservoir Apertures of Different Sizes

Release Aperture, Side Dimension (μm)	Mean Time to 50% Recovery (min)	SD of Mean Time to 50% Recovery (min)	Replicates
50	216	31	5
200	125	28	4

lyophilized leuprolide in a solid PEG matrix relative to solution phase leuprolide. Presumably the stability advantage offered by solid dosage forms will be greater with more labile drugs.

In Vitro Release Kinetics and Reservoir Geometry

Release kinetics can also be controlled by changing the reservoir geometry, especially the area of the opening through which the release occurs. The pyramidal reservoirs in the silicon layers of the microchips (Fig. 2) are created with an anisotropic wet alkaline (potassium hydroxide) etch of the silicon wafer (14). The dimensions of the release aperture for each reservoir can be controlled by varying the area of the wafer that is exposed to the etchant solution and the duration of the etching process.

A series of microchips were fabricated with aperture dimensions of 50 μm × 50 μm and 200 μm × 200 μm. The reservoirs were filled with an aqueous solution of leuprolide acetate (260 mg/ml), the solutions were lyophilized, and the void volumes of the lyophilized cakes were displaced with (molten) PEG 1450, to yield a solid-in-solid matrix formulation. The same leuprolide filling solution was used for all filling activities. The chips were sealed and *in vitro* release experiments were performed, as described. The results are graphed in Fig. 9 and measured release properties are listed in Table III. Quantitative mass recoveries were obtained for all releases, and the observed release rates increase with increasing aperture size, as expected.

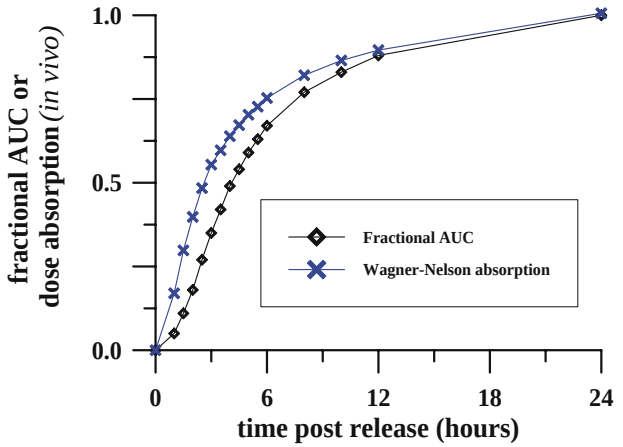

Fig. 11. Fractional AUC for *in vivo* release of leuprolide from implanted reservoir arrays and the fractional absorption of leuprolide, calculated from serum PK data using the Wagner–Nelson equation (k_{el} 0.73 hr^{-1}), as a function of time post release activation.

In Vitro–In Vivo Correlations

The average *in vivo* serum leuprolide level following the release of a formulation of lyophilized leuprolide in a solid matrix of PEG 1450 from multi-reservoir arrays implanted in beagle dogs is presented as a function of time following the release activation in Fig. 10 (10). The fractional area under the serum PK curve is presented in Fig. 11 as a function of time post release activation. The serum PK data was also used to calculate fractional dose absorption by application of the Wagner–Nelson equation. The elimination rate constant, k_{el}, of the Wagner–Nelson equation can be obtained by regression analysis on the later time points of a PK profile, assuming that at later time drug is no longer entering the

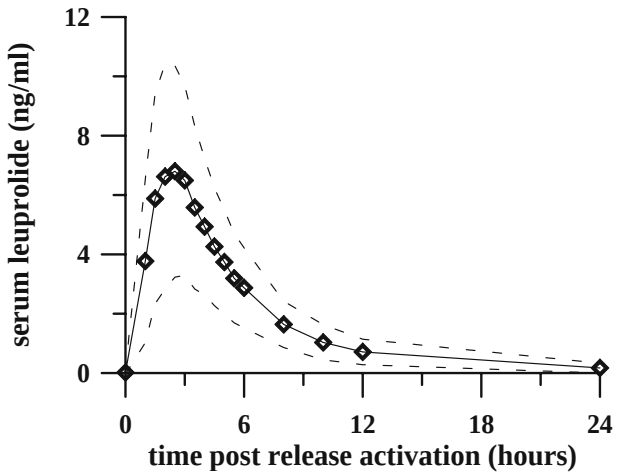

Fig. 10. Average *in vivo* release profile for a solid dosage form of leuprolide released from implanted reservoir arrays in the subcutaneous space of beagle dogs (10). The *dashed lines* represent +/−1 standard deviation of the mean ($n = 68$).

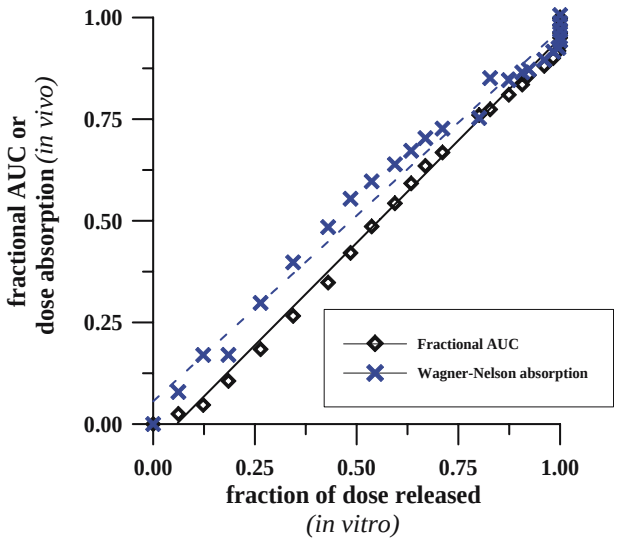

Fig. 12. In vitro–in vivo correlation for the leuprolide release data of Figs. 7 and 11.

Table IV. Summary of the Fit Parameters for the IVIVCs Plotted in Fig. 12

In Vivo Data Set	Slope	Intercept	R^2
Fractional AUC	1.01	−0.06	0.99
Wagner–Nelson absorption (k_{el} 0.73)	0.91	0.06	0.99

blood compartment from the injection site or peripheral compartments. This is problematic for subcutaneous injections and controlled release formulations; drug may still be entering the circulation from the injection site or peripheral compartments at earlier time points, and the signal intensity is weaker and more variable at the later times. In the present work k_{el} was calculated from published serum PK data for an intravenous bolus of leuprolide in beagle dogs (15). The calculated value (0.73 hr^{-1}) was used in the Wagner–Nelson equation to determine absorption kinetics from the serum PK data for leuprolide released from the implanted multi-reservoir arrays. The fractional dose absorbed as calculated from the Wagner–Nelson relationship is presented with the fractional AUC in Fig. 11. The IVIVCs obtained from the comparison of interpolated *in vitro* and *in vivo* data sets are presented in Fig. 12, and the fit parameters are listed in Table IV. The correlations are excellent (R^2 of 0.99) with slopes near unity, demonstrating level A-type IVIVCs for the pulsatile release of the solid formulation of leuprolide. This suggests that the dissolution and diffusion controlled release of leuprolide from the implanted reservoir arrays was the rate limiting step in the *in vivo* absorption, and that the rate of leuprolide transport through encapsulating tissue was faster than the rate of release from the reservoirs. This is an encouraging result regarding the use of the implanted multi-reservoir arrays for *in vivo* drug delivery applications, and suggests that the rate of drug absorption could be accelerated by increasing the rate of release from the reservoirs.

CONCLUSIONS

Novel drug delivery and biosensing devices have the potential to increase the efficacy of drug therapy by providing physicians and patients with the ability to precisely control critical therapeutic parameters. Such "intelligent" systems can enable control of dose amount and the time, rate, and location of drug delivery. Implanted multi-reservoir arrays, such as the microchip-based array used in this work, can be attractive alternatives to drug delivery by injection. Controlled release of the polypeptide leuprolide acetate demonstrates advantages of this approach for drug delivery: the ability to store and release discrete solid phase formulations, which may be inherently more stable than solution phase formulations, and the ability to perform the release(s) on demand. Polypeptide solutions can be lyophilized on the microchips, enhancing long term stability at 37°C as a consequence of low moisture content, which can be maintained in moisture-tight sealed reservoirs.

A successful drug delivery device must provide performance advantages that offset potential shortcomings of implants. The relatively small volume per unit dose is one limitation of the present multi-reservoir array microchip design. These studies demonstrate successful preparation of high drug concentration formulations, alleviating the volume limitation. Although reservoir filling is performed with solutions, post-fill processing such as lyophilization eliminates the need for long-term solution phase drug stability. The microchip may be redesigned, using proven device fabrication approaches that can be applied to commercial manufacture, to increase the reservoir volume by a factor of 3 or greater without changing release activation power requirements or reservoir packing density on the microchip.

Therapeutic agents approved for marketing or in late stage development which are not orally bioavailable, and which are sufficiently potent that an efficacious dose volume will be compatible with the volumetric constraints of our current reservoir design, include FORTEO™ [teriparatide (rDNA origin) injection] for the treatment of osteoporosis, BYETTA™ (exenatide) injection for the treatment of type 2 diabetes mellitus, PRIALT® (ziconotide intrathecal infusion) for pain management, and bone morphogenic proteins (BMPs) for stimulating bone growth. We have successfully prepared lyophilizable solution phase forms of parathyroid hormone, including aqueous solution phase teriparatide (hPTH(1–34)) at concentrations exceeding 400 mg/ml, with sufficient stability to permit chip filling and lyophilization. We have also performed *in vitro* releases of clinically efficacious doses of hPTH(1–34) from single reservoirs.

Implanted drug delivery systems will play a growing role in realizing the therapeutic potential of peptides and proteins. We have previously described the controlled pulsatile release of a novel formulation of the polypeptide leuprolide from microchip implants, each containing an array of discrete doses, in a canine model for nearly 6 months (10). This was the first demonstration of chronic, programmed delivery of therapeutic macromolecules from wireless microchip implants. The strong correlation between the rates of *in vitro* release and *in vivo* absorption for a solid dosage form of leuprolide released from the implantable arrays suggests that the pharmacokinetics for this drug-device combination are controlled by the rate of release from the device. It should therefore be possible to achieve faster absorption, and possibly injection-like pulsatile pharmacokinetics, by providing faster release from the reservoir. Future development activity with the multireservoir array will focus on achieving pharmacological equivalence with subcutaneous injection. Other variables that may be tailored to meet application specific requirements include reservoir geometry and the size and shape of the reservoir array. Novel non-thermal reservoir sealing techniques that provide hermetic seals and the storage of discrete doses under reduced pressure have been demonstrated. The ability to achieve hermetic seals at ambient temperatures and under vacuum will improve drug stability and eliminate the need for the post-lyophilization void-volume displacing fill. Future studies will also investigate biosensing applications of multi-reservoir arrays.

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