

Feasibility of Formulating hPTH(1-34) for Delivery from a Multi-Reservoir Array

Elizabeth R. Proos; MicroCHIPS, Inc, 6-B Preston Court, Bedford, MA 01730 (www.mchips.com)
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Abstract

Purpose. Implantable drug delivery devices can control delivery of multiple doses for chronic therapy. For example, osteoporosis patients could avoid daily injections over an 18 month course of parathyroid hormone therapy if drug were delivered via an implant. Formulation screening experiments were conducted to test the feasibility of preparing highly concentrated human parathyroid hormone (1-34) (hPTH(1-34)) for delivery from individual reservoirs of such an implantable micro-reservoir array (ca. 300nL/reservoir). An acceptable device would need to deliver a therapeutic dose (20 µg) as a pulse. Formulation constraints include peptide solubility, dissolution rates in an isotonic (adjusted with sodium chloride) neutral (pH 7.4) phosphate buffer (PBS), and stability at 37°C.

Methods. Concentrated aqueous hPTH(1-34) solutions (50-250mg peptide/mL) were prepared. Dissolution- and stability-promoting excipients included acetic acid, citric acid, histidine, and trehalose. The solutions were lyophilized as 20µL aliquots in vials for stability determination at 37°C or were filled in reservoirs (200nL per reservoir) and lyophilized to determine *in vitro* release properties. The hPTH(1-34) concentration and purity were determined by reverse phase HPLC. The dissolution time was recorded when stability samples were recovered in PBS. *In vitro* release testing was performed from multi-reservoir arrays in PBS, using a custom flow cell and related apparatus.

Results. Inclusion of histidine and citric acid in the formulations yielded lyophilisates which were stable (>55 days at 37°C; >90% purity) and dissolved rapidly in PBS. *In vitro* release testing resulted in high yields (90% recovery) and pulsatile releases ($t_{1/2} \leq 3$ hours). In the absence of citric acid the *in vitro* release of hPTH(1-34) was slow and incomplete (<20% recovery, $t_{1/2} > 24$ hours).

Conclusions. Stability testing indicates the feasibility of preparing highly-concentrated hPTH(1-34) lyophilisates that will be sufficiently stable for extended storage at 37°C. Inclusion of a non-volatile pH modifier (citric acid) in hPTH(1-34) lyophilisates provided a low pH micro-environment which facilitated rapid and complete peptide dissolution on contact with PBS. These acidic dosage forms enabled pulsatile release from a micro-reservoir of an implantable drug delivery device to an external PBS solution.

Core Technology

The multi-reservoir microchips were prepared using established microfabrication methods (Maloney, J.M. *et al.* (2005) *J. Control. Release* **109** 244-255). Each 10 mm x 10 mm microchip consisted of a silicon and glass bonded substrate containing 24 individually addressable, 300 nL-capacity reservoirs (Figs. 1 and 2). Metal membranes composed of platinum and titanium layers (40 nm Pt/ 300 nm Ti/ 40 nm Pt) hermetically sealed the drug-releasing opening of each reservoir. The membranes were electrically connected to the device's control electronics through circuit traces on the microchip. To expose the contents of a specific reservoir, a current pulse (~1 A) was applied to the membrane, causing local heating and disintegration of the membrane within 10 µs (Fig. 3). This device design and activation method enables specific reservoirs to be addressed and opened on-demand, provides nearly instantaneous activation in any environment (*i.e.*, not limited to saline solutions), and allows the verification of release activation by measuring circuit resistance.

Project Objectives

Criteria

- Clinically relevant dose per reservoir (Forteo™ dose is 20µg once a day)
- Stable at 37°C
- Narrow delivery window (mimic once daily injection of Forteo™ - peak serum concentration in 30 minutes, ideally returning to baseline in ≤ 3 hours)

Challenge

- Small reservoir volume (300nL means ≥ 67 mg/mL starting concentration; Forteo™ is formulated at 0.25mg/mL)
- Most peptide and protein formulations are developed for stability at temperatures < 30°C
- Limited solubility of hPTH (1-34) in physiologic conditions

Approach

- Formulate in acid where hPTH (1-34) is highly soluble (>400mg/mL)
- Lyophilize the formulation and include stabilizing excipients
- Include a pH modifier to create a local pH gradient upon release activation

Multi-Reservoir Array Platform

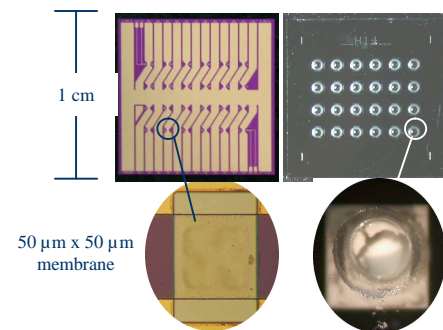


Figure 1: Membrane and fill side images of a multi-reservoir array

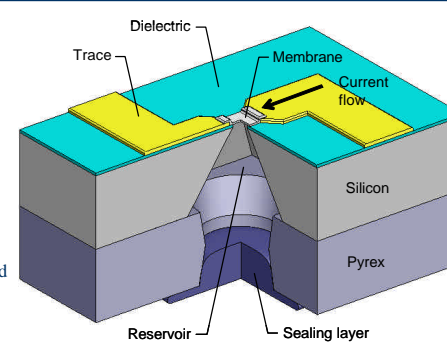


Figure 2: Cross-sectional representation of a single reservoir from the multi-reservoir array.

- Each reservoir membrane/ fuse is part of an electric circuit.
- Capacitor discharge through the fuse causes resistive heating of the membrane (1 A and 6 V for a 50 µm x 50 µm opening).
- The membrane/ fuse ablates within 10µs, activating release by exposure of the reservoir contents.
- The electronics provides the ability to address specific reservoirs for release initiation, and for confirmation of release initiation by measuring circuit resistance.

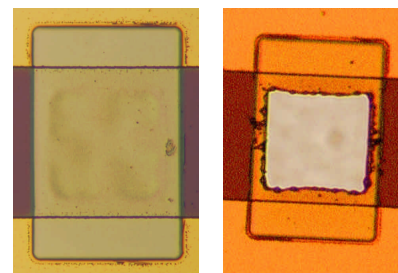


Figure 3: Individual unopened membrane (left) and opened (right, back-lit).

Methods

Formulation preparation: Concentrated solutions of hPTH (1-34) were prepared at room temperature and dispensed as either bulk (20µL droplet pipetted into a 2mL glass serum vial) or on-chip (200nL dispense per reservoir using a custom filling instrument equipped with a 32 gauge needle) samples. Bulk and on-chip samples were lyophilized using a commercial shelf freeze dryer (VirTis, Inc., Advantage EL) (Fig.4). Bulk samples were used for dissolution and stability testing (capped under argon and stored at 37°C). On-chip samples were used for *in vitro* release testing.

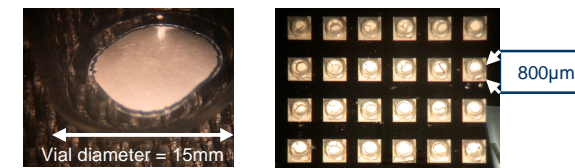


Figure 4: Bulk lyophilisate (2mg hPTH (1-34) in a glass vial) (left) and on-chip lyophilisates (20µg hPTH (1-34) per reservoir) (right).

Bulk dissolution and stability testing: Immediately after freeze-drying or after storage at 37°C, lyophilisates were photographed and recovered in 10mM sodium phosphate pH 7.4, 140mM sodium chloride, 2.7mM potassium chloride, 0.004% Tween-20 (PBS), by adding a known volume of buffer to the vial with minimal agitation. Observations about dissolution were recorded. Dissolved samples were tested for purity and recovery.

Peptide concentration and purity determination: Samples were tested for recovery (% total area compared to theoretical) and purity (% main peak area compared to total area) using a reversed phase HPLC (RP-HPLC) method (Agilent 1100 Series, Vydac C18 column, 2.1 mm x 15 cm, 215 nm detection). A gradient method was employed using 0.2% trifluoroacetic acid and a flow rate of 0.2mL/min throughout: 0 to 3 minutes: 82% water:18% acetonitrile; 3 to 54 minutes: linear gradient to 58% water:42% acetonitrile.

***In vitro* release (IVR) testing:** A filled, lyophilized, sealed microchip was mounted on a circuit board and assembled in a custom flow cell apparatus. A cable provided communication from the board interface to a computer program developed at MicroCHIPS that controlled the selection of number and identity of reservoir membranes. The flow cell directed the PBS wash solution over the membrane side of the chip face. A precision metering pump controlled the flow of wash solution into the flow cell inlet ports, over the chip face, through outlet ports, and into a fraction collector (Fig. 5). No flow occurred across the chip face during activation (opening) of the membranes and between collection time points. The entire assembly, including buffers, was maintained at 37°C. Peptide was quantitated in fractions using the above RP-HPLC method. Four reservoirs (each containing 20µg peptide) were opened per release event to maintain a fraction concentration level above the limit of quantitation.

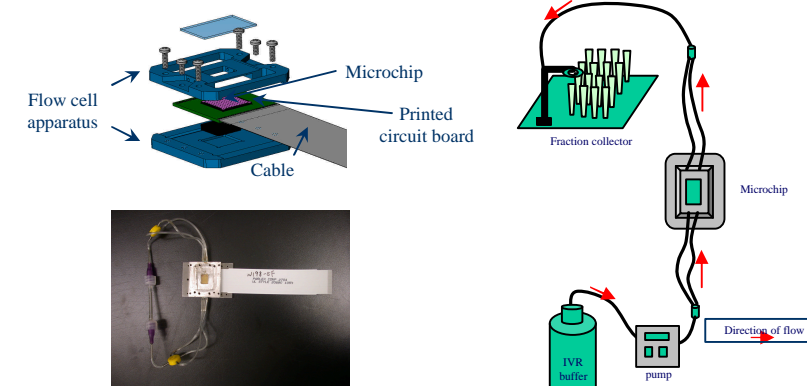


Figure 5: Schematic and photograph of IVR flow cell apparatus (above) and schematic representation of IVR set-up (right) (NOT to scale)

Results

Bulk dissolution and stability

The hPTH (1-34) used in these experiments is at least 98% pure as received, according to manufacturer specification. Purity results are presented as % purity retained compared to the starting material. To illustrate the progress of significant formulation improvement during development, selected examples are provided (Fig. 6). Each data set consists of the average of three samples. The most stable formulation prepared to date retains 95% purity (2% RSD) with 99% recovery (8% RSD) after storage as a lyophilized solid at 37°C for 55 days. Recovery was performed in PBS at room temperature.

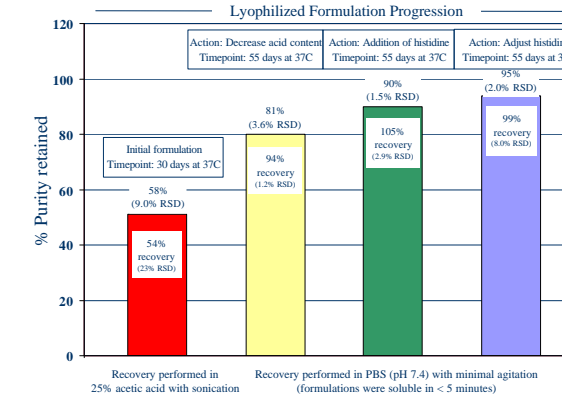


Figure 6: % purity of high concentration (60-200mg/mL) lyophilized hPTH (1-34) formulations after storage at 37°C for 55 days. (While the concentration range may seem large, it is feasible to use any formulation in this range to achieve a therapeutic dose per reservoir.)

In vitro release

In vitro release testing was conducted to determine release reproducibility and release kinetics (pulsatile) of an hPTH (1-34) formulation from a multi-reservoir array. Release from the reservoir, once the membrane is opened, is controlled by the dissolution and diffusion of the formulation from the reservoir. Reproducible pulsatile release was demonstrated with signal returning to baseline in ≤ 3 hours and with $T_{1/2} = 15-18$ minutes (Figs. 7 and 8). MicroCHIPS' formulation exhibits significantly improved dissolution properties compared to the starting material (Fig. 9).

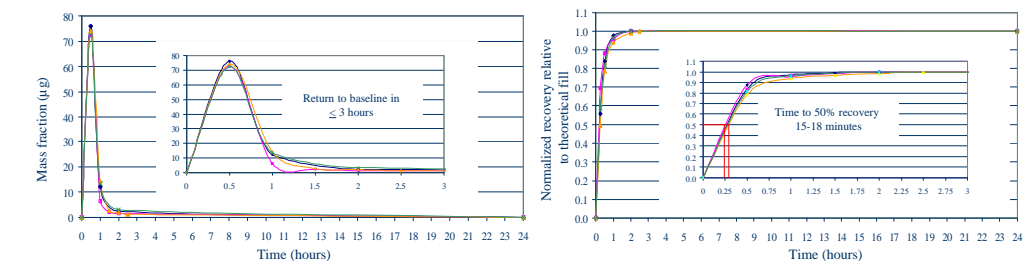


Figure 7: Four independent release events (3-1st chip, 1-2nd chip) were performed showing the mass of hPTH (1-34) collected per fraction (left) and the normalized recovery relative to theoretical fill to determine the $T_{1/2}$ (right).

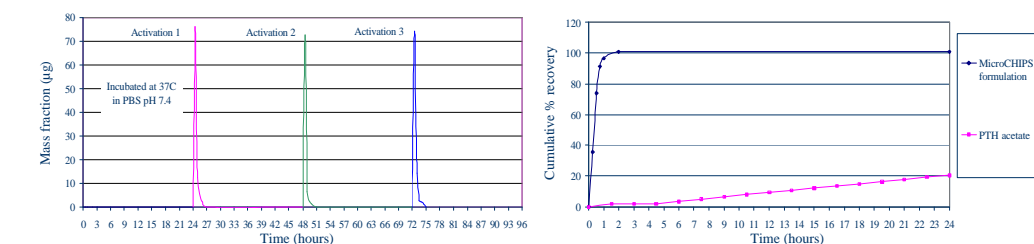


Figure 8: Three consecutive release events from a single chip demonstrate the pulsatile release properties of this device/ formulation combination.

Figure 9: Illustration of solubility limitations of PTH acetate without enhanced formulation: pulsatile release of MicroCHIPS' formulation vs. steady state release of PTH acetate

Conclusions

This project demonstrates the feasibility of reproducibly releasing therapeutically relevant doses of polypeptides (*i.e.*, hPTH (1-34)) from implantable multi-reservoir arrays, even when indications (*i.e.*, osteoporosis) require precisely controlled dose timing and pulsatile release kinetics. While not optimized, reasonable stability of a highly concentrated formulation was achieved for 55 days at 37°C through rational formulation design. The multi-reservoir drug delivery platform has a number of advantages over existing controlled release technologies, including long-term protection of formulations from physiological fluid, and the ability to maintain discrete solid phase doses.

Acknowledgements

Many MicroCHIPS employees have contributed to the demonstration of feasibility of drug delivery from multi-reservoir arrays by creating and continuously improving this technology. Special recognition is given to Jim Prescott and Mark Staples for their contributions to this project.