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In Vivo rapid delivery of vasopressin from an implantable drug delivery micro-electro-mechanical device

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Abstract

A miniaturized implantable rapid drug delivery device based on micro-electro-mechanical-systems technology was recently developed and characterized. This device is intended to address acute conditions in high-risk subjects. This work provides an in vivo proof-of-concept for the device in a rabbit model, by releasing a physiologically active dose of vasopressin, a vasoconstrictor. The devices were implanted subcutaneously and activated to rapidly release vasopressin, with monitoring of mean arterial pressure and plasma levels.

Device releases showed a rapid and measurable effect on mean arterial pressure as well as a continuous diffusion of vasopressin into the bloodstream, consistent with a depot effect. Plasma levels in rabbits receiving vasopressin with the device rose monotonically to 24.4 ± 2.9 ng/mL after one hour. Bioavailability after one hour was calculated to be 6.2 ± 2.8 % (mean ± s.d.).

A new modality for rapid and controlled drug delivery has been developed. The device can be used as a new implantable device controlled by medical algorithms (based on heart rate or mean arterial pressure, for example) for autonomous operation in high-risk populations that require immediate ambulatory intervention.

Keywords: Subcutaneous drug delivery; vasopressin; MEMS; rabbit; bioavailability.

Introduction

MEMS technology has allowed the implementation of implantable drug delivery systems for applications that typically require sustained plasma drug levels for treatment of chronic illnesses. A number of implantable drug delivery devices have been investigated for use in chronic and non-chronic diseases in ambulatory settings without medical intervention, such as cancer, diabetes and osteoporosis[1-4]. Drug delivery devices characterized by multiple-reservoir architectures and based on electrical actuation mechanisms, for example, provide a reliable platform because of the reproducibility of MEMS technology. These devices typically rely on electro-thermal actuation to rupture a reservoir-sealing membrane as a result of an applied electrical potential, allowing the drugs inside the reservoirs to freely diffuse into the region of interest[2,5]. Such implantable devices provide a significant improvement in the bioavailability of drugs with poor oral bioavailability and alleviate problems linked to patient compliance. Their use is however limited to treatment of chronic illnesses as they rely on diffusion and small delivery volumes, with complete drug release achieved in the range of hours to days.

The Implantable Rapid Drug Delivery Device (IRD^3) was previously reported in vitro to deliver a volume of 20 μL in 45 seconds[6]. The delivery mechanism relies on the rapid creation and expansion of vapor bubbles within the device to force the drug solution out of the device. This mechanism is similar to thermo-pneumatic micropumps, with an important difference. Thermo-pneumatic micropumps typically achieve pumping by displacing an elastic membrane against the fluid to be delivered. Expansion and cooling of a gas or fluid in a separate chamber is achieved through microresistors [7-9]. The flow rate can be controlled by modulating the voltage applied to the microresistors and the duty cycle, typically reaching a few microliters per minute. The IRD^3 is characterized by its small size and a faster actuation mechanism, allowing rapid drug release. This device is of particular importance for emergency therapies requiring immediate drug delivery in high-risk patients who suffer from conditions with life-threatening acute symptoms.

Vasopressin is a peptide found in most mammals. It serves two specific purposes: the modulation of water retention in the kidneys in response to increased osmolality in the blood, and at higher concentrations, the vasoconstriction of arterioles to raise blood pressure in the event of hypotension caused by hemorrhage[10-13]. It was chosen as the model drug because it is widely used as vasoconstrictor in cardiac arrest[14,15] and shows promise in the treatment of hemorrhagic shock[16-19]. It is thus a candidate for release from our device in this time-sensitive context.

One potential application of this device with vasopressin is the ambulatory treatment of wounded soldiers at risk for hemorrhagic shock. The device would be preventatively implanted in a soldier before leaving for the front. The triggering of this device would be controlled by a medical algorithm evaluating blood pressure and heart rate against pre-defined thresholds. This data would be obtained from standard wearable sensors. Hostile conditions can delay medical treatment on a battlefield and evacuation conditions are often less than ideal to administer therapy by injection, but in the case of hemorrhagic shock, the window of opportunity for treatment can be on the order of minutes. A subcutaneous device triggered automatically or remotely to deliver a dose of vasopressin could help sustain a soldier's blood pressure during transportation, delaying the onset of hemorrhagic shock until proper medical treatment can be administered in more controlled conditions, e.g., a field hospital.

The objective of this work was to demonstrate in vivo operation of the IRD^3 by releasing a physiologically active dose of vasopressin and measuring its effects on blood pressure in rabbits. This would provide a proof of concept for in vivo rapid drug delivery from an implantable device.

Material & Methods

Vasopressin and Devices

Arginine vasopressin acetate powder (Sigma-Aldrich, Saint-Louis, MO) was reconstituted with sterile water to appropriate concentration for injection or device loading. Device fabrication and drug loading have been extensively described previously[6].

Animal Study

This study was approved by MIT's Committee on Animal Care and the Animal Care and Use Review Office from the Department of Defense Medical Research and Material Command. Sixteen male New Zealand White rabbits with weights between 2.5 kg and 3.5 kg were obtained (Covance Research Products, PA, USA). The animals were housed in compliance with MIT's Committee on Animal Care husbandry guidelines, with unrestricted access to water and food.

Rabbits were anesthetized with an initial dose of midazolam (2 mg/kg, IV) and were maintained under anesthesia by mask with 1-3% isoflurane in balance oxygen. The central auricular arteries in

both ears were catheterized for mean arterial pressure (MAP) monitoring and blood sampling, respectively. MAP was monitored continuously by direct blood pressure measurement from an arterial catheter in the central auricular artery using a blood pressure sensor (Blood Pressure Analyzer™ 400, Micro-Med, Louisville, KY), and recorded with the DMSI Software (Micro-Med). Values were measured every 0.5 seconds and averaged over 10 seconds to yield a single data point.

The rabbits were randomly assigned to receive 100 µg/(kg body weight) vasopressin subcutaneously (1 mL, conc. 0.25-0.35 mg/mL, Control group, n = 5), an IRD[3] containing 20 µL of solution with the same total dose of 100 µg/(kg body weight) vasopressin (conc. 12.5-17.5 mg/mL, Device group, n = 3), a subcutaneous injection of 1 mL water (Placebo Injection group, n = 4), or an IRD[3] containing 20 µL of sterile water (Placebo Device group, n = 4). The devices were implanted subcutaneously and activated immediately after connection to the external power source.

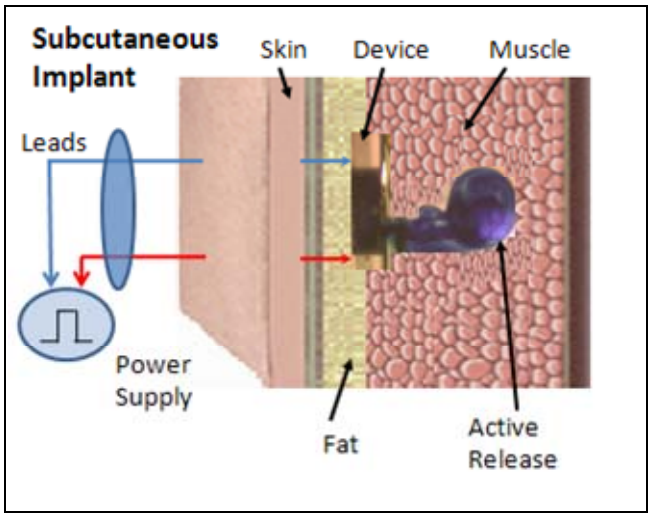

Figure 1: Schematic representation of the experimental procedure.
A device, 9 mm (L) x 6 mm (W) x 3 mm (H), is implanted subcutaneously and connected to an external power source. Activation of the device forces the drug solution out of the device.

Figure 1 shows a schematic overview of the experiment. A 1-cm skin incision was made across the left triceps of the anesthetized rabbit, approximately 2 cm from the elbow. A subcutaneous pocket was created by blunt dissection over the triceps. The device was introduced into the pocket through the incision, with the drug exit side facing the muscle. Biocompatible Teflon-sheathed electrical leads were used to connect the microheaters to the external power source through the incision. Devices were activated by applying 9 V to the microheaters for 10 seconds, followed by a 15 second pause and ten 5-second applications of 9V with 5-second intervals, in order to minimize heat exposure to the animal and drug degradation due to activations. Membrane rupture was monitored by measuring the resistance of a metallic line patterned over the membrane for circuit opening. Injections were performed at the same subcutaneous location where a device would be implanted, and the solution was injected over a period of approximately 2 seconds, as would be done in standard clinical practice. MAP was continuously monitored for 1 hour following injection or activation. The animals were euthanized at the end of the monitoring period.

MAP Data Extraction

MAP data was normalized to the starting pressure by subtracting the average MAP during the 5 minutes preceding the injection or device activation. The normalized data (ΔMAP) for each animal receiving vasopressin was analyzed (Excel 2003, Microsoft, Inc., USA) to extract the maximum ΔMAP (ΔMAP_{max}), the time needed to reach that maximum (t_{max}), and the time at which ΔMAP returned to less than zero for at least 1 minute thereafter (t_{dur}). The normalized data for each animal in a placebo group was analyzed to extract ΔMAP_{max} only. This number represents the maximum random positive fluctuation in MAP experienced during the monitoring period. There is thus no meaning in the values of t_{max} and t_{dur} for the placebo groups.

Plasma Levels and Bioavailability of Vasopressin

Three rabbits in the Control and Device groups received solutions containing ^{3}H-radiolabeled vasopressin (American Radiolabeled Chemicals, St. Louis, MO). One blood sample was collected before injection as control and up to 7 samples after injection at chosen time points up to 1 hour.

The drawn blood samples were collected into BD Vacutainer® tubes coated with sodium heparin (VWR, West Chester PA) and immediately refrigerated for further processing. Samples collected within one day were centrifuged at the end of each day to collect plasma. Plasma samples were then processed per the manufacturer's instructions using SOLVABLE™ (PerkinElmer, Waltham, MA) and counted in a liquid scintillation counter (Packard Tri-Carb 2200CA, PerkinElmer, Waltham, MA) to determine plasma levels.

The bioavailability F of a drug delivered by an experimental route or formulation is expressed by:

$$F = \frac{AUC_{\exp} * Dose_{ref}}{AUC_{ref} * Dose_{\exp}} \quad \text{(Eq. 1)}$$

AUC is defined as the area under the plasma level versus time curve of the tested drug using the trapezoidal rule, up to the last collected data point [20]. The reference sample is generally an intravenous dose of the drug, as it allows all of the injected drug to be exposed to the body. Each AUC is normalized by the given dose for comparison. AUC was computed for each animal and averaged within each group (n = 3 for each group). The reference sample consisted of three animals that were given an intravenous injection of vasopressin. The AUC for each reference animal was also computed and the three values were averaged together to yield AUC_{ref} (data not shown). The bioavailability after one hour for the Control and Device groups was then calculated using Equation 1.

Statistics

Extracted values for each group were analyzed for statistical significance with Student's t-test. A p-value lower than 0.05 was considered to be statistically significant. The ΔMAP_{max} of each vasopressin group was compared to its corresponding placebo group, in order to evaluate whether the changes happening after vasopressin delivery were solely due to random fluctuations in MAP. Additional comparisons were made at salient time points to contrast the Control and Device groups with each other and with their respective placebo groups.

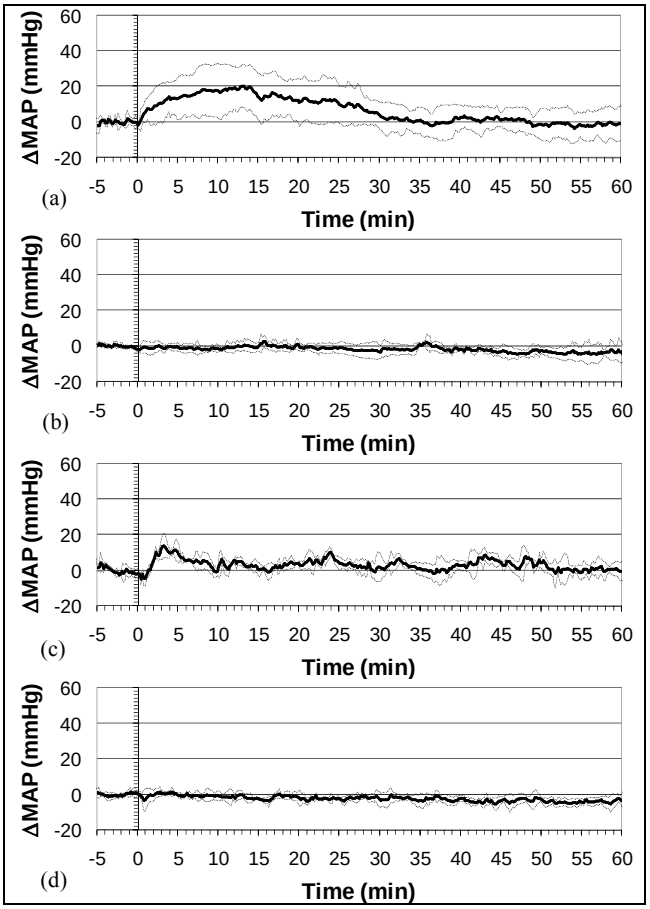

Figure 2: Average ΔMAP profile for all groups.
(a) Control (n = 5), (b) Placebo Injection (n = 4), (c) Device (n = 3), and (d) Placebo Device (n = 4). Dotted lines represent ± 1 standard deviation.

Results

Figure 2 shows averaged ΔMAP profiles depicted in bold and their standard deviations in dotted lines. Figure 2(a) presents the profile for the Control group, showing an immediate increase in

MAP to reach a maximum after 14 minutes, followed by a steady decrease to baseline at t = 35 minutes. Figure 2(b) shows the profile for the Placebo Injection group, which presents no feature, beside random fluctuations. A t-test between the two groups at t = 14 minutes yielded a p-value of 0.02, suggesting the effect on MAP from vasopressin injection was significantly greater than from the water injection. Figure 2(c) shows the results for the Device group. All devices opened within 10 seconds after the start of activation. The MAP reaches a peak in 4 minutes, followed by a significant return to baseline (> 1 minute) at t = 35 minutes. This decrease is not monotonic, however. Smaller peaks are experienced at t = 24 and 32 minutes. There is also a slight dip in MAP within the first 2 minutes. Figure 2(d) represents the results for the Placebo Device group, and shows the same dip within the first 2 minutes, but no other notable feature. A t-test at each of the peaks observed in Figure 2(c) (t = 4, 24 and 32 min) revealed that these peaks were significantly different from the placebo effects at their respective time points (p = 0.0004, 0.001 and 0.01, respectively).

Table 1: Summary of ΔMAP_{max}, t_{max} and t_{dur} for each test group. Values are reported as mean ± s.d.

Group	ΔMAP_{max} (mmHg)	t_{max} (min)	t_{dur} (min)
Control (n = 5)	24.3 ± 11.5	12.0 ± 4.6	39.1 ± 23.0
Placebo Injection (n = 4)	5.5 ± 2.3	---	---
Device (n = 3)	17.2 ± 4.2	4.0 ± 0.9	33.9 ± 6.0
Placebo Device (n = 4)	4.7 ± 0.7	---	---

The measured data extracted from the results is summarized in Table 1. The Control and Device groups were found to reach a ΔMAP_{max} significantly higher than their respective placebo (p = 0.02 and 0.03, respectively). This means that the effects observed in both vasopressin groups are significantly greater than could be observed from any random fluctuation in MAP. The Device and Control groups were also compared. ΔMAP_{max} and t_{dur} were not significantly different (p = 0.26 and 0.65, respectively), but t_{max} was (p = 0.02). Further contrasting of the two groups using time as a parameter found that at t = 4.0 minutes (t_{max} for the Device group), ΔMAP was not significantly different (p = 0.61), but at t = 14.0 minutes (t_{max} for the Control group), ΔMAP for the Control group was significantly higher (p = 0.04).

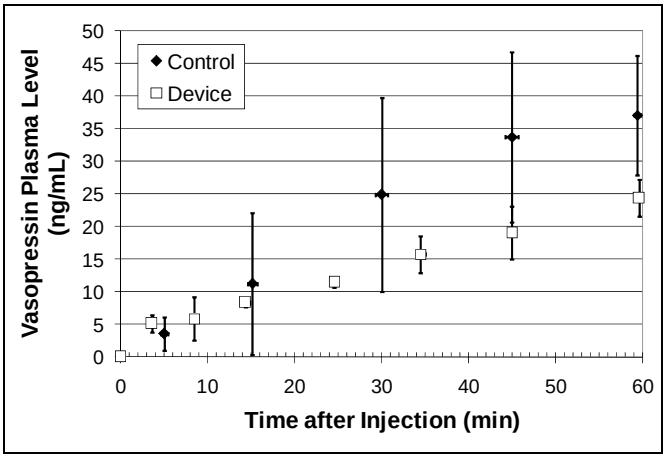

Figure 3: Vasopressin plasma levels measured by liquid scintillation counting.

Error bars represent standard deviation. n = 3 for both groups, except for the Device group where a blood sample could not be obtained at 15 minutes for one rabbit and at 25 minutes for another rabbit. n = 2 at those points.

Figure 3 shows measured plasma levels for the Control and Device groups. Plasma levels in both groups rose monotonically up to one hour after injection or activation, but at different rates. Plasma levels in the Control group increased linearly in the first 45 minutes at a rate of 0.77 ng/(mL-min), after which the rate of increase slowed down to reach a maximum concentration of 37.1 ± 9.2 ng/mL one hour after the injection. Plasma levels in the Device group initially rose to 5.1 ± 1.3 ng/mL after 3.6 minutes, but increased at a slower rate of 0.35 ng/(mL-min) thereafter to reach a final concentration of 24.4 ± 2.9 ng/mL

after one hour. Bioavailability after one hour was calculated to be 10.0 ± 6.3 % and 6.2 ± 2.8 % (mean ± s.d.) for the Control and Device groups, respectively.

Discussion

This work has shown that vasopressin delivered subcutaneously was able to quickly achieve plasma levels sufficient for vasoconstriction. An injection of 100 □g/kg resulted in an immediate response, as evidenced by the MAP increase shown in Figure 2(a). A MEMS drug delivery device implanted subcutaneously was also able to deliver the same vasopressin dose with an immediately measurable effect. The overall response was lower with delivery from the device, however, as suggested by the lower overall MAP profile and statistical analysis at the peak of the Control group. This work nonetheless represents the first time to the authors' knowledge that a drug released from an implantable drug delivery device based on MEMS technology achieved significant plasma levels with measurable physiological effects within this short period of time.

There are a few potential causes behind the different MAP response of device delivery compared to syringe injection. The first is an in vivo behavior of the devices different from in vitro experiments regarding released fraction and stability of the solution. Previous in vitro experiments determined that device activation resulted in degradation of 9% of the released vasopressin [6]. Quantification of the in vivo degradation of vasopressin after activation was not feasible because of the impossibility in collecting the released solution in the subcutaneous pocket after activation. The authors can only conclude that although more significant degradation may have happened than in vitro, a sufficient amount of vasopressin remains to affect the vascular system. The activation of the device, whether thermal or electrical, may have also slightly affected the animals, exhibited as small dips in MAP observed with the Device and Placebo groups during the first 2 minutes. The difference in the amount of fluid delivered (20 □L vs. 1 mL) can also result in reduced exposure to the bloodstream, which may contribute to the observed lower bioavailability of the device release. This issue, however, can be addressed by adjusting the delivered dose. Finally, the rapid decrease in the rate of increase of vasopressin levels suggests that the very high concentration of vasopressin in the device (approximately 50 times higher than that of an injection) may be subject to a rate-limiting step. The transition between tissue and blood vessel may be such a rate-limiting step, for example, especially coupled with the fact that vasopressin itself constricts the blood vessels, further reducing the cross-sectional area available for diffusion.

It is not known whether the smaller peaks observed at t = 24 and 32 minutes are related to issues of degradation or diffusion. Further experimentation would be needed to shed a light on this issue.

One of the advantages of the device over syringe injection is made apparent by the smaller standard deviation of the MAP profile, even with a larger control sample size. This observation highlights the fact that device delivery results in more consistent behavior than syringe injection, in addition to the fact that device opening was achieved every time within 10 seconds after activation. Subcutaneous injections can be difficult to perform consistently, especially in suboptimal conditions (e.g. ambulatory settings). One only has to refer to the literature on subcutaneous administration of insulin in the management of diabetes to see that subcutaneous injections cannot ensure repeatable pharmacokinetics [21-23]. A device previously placed in the appropriate location in high-risk subjects will ensure repeatable and rapid delivery. This device can furthermore be coupled with receiving and triggering electronics, resulting in a remotely activated device, or if coupled with a blood pressure sensor and the appropriate medical algorithm, could become an autonomous

system, triggering automatically when blood pressure reaches critical levels.

It has been previously reported that the pharmacokinetics of vasopressin in New Zealand White rabbits are biexponential, reflecting two vasopressin elimination processes. The faster process is easily saturated and has reported half-lives between 0.6 and 0.9 minutes, while the slower process has reported half-lives between 5 and 6 minutes [24]. Such short half-lives imply that more than 90% of intravenously injected vasopressin is eliminated from the bloodstream after 15 minutes. Intravenous injections of vasopressin in rabbits (not shown) confirmed that the plasma levels of vasopressin fall to less than 10% of their nominal original level 15 minutes after injection. The monotonically increasing vasopressin levels measured in both the Control and Device groups for 1 hour thus indicate the presence of a depot effect, with vasopressin entering the bloodstream at a higher rate than it is eliminated. It is thus counter-intuitive to see MAP decrease in both cases while plasma levels of vasopressin are still increasing. The observed discrepancy between blood pressure and plasma level is similar to that observed by Dworkin et al, who reported a loss of vasoconstriction after 15 minutes of a 30-minute infusion of vasopressin directly to the hepatic arterial bed in rats [25]. Co-infusion of vasopressin with the nitric oxide (NO) inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) demonstrated that this loss of vasoconstriction was tied to the triggered production of NO, which caused vasodilation. The continued delivery of vasopressin in a healthy animal to induce forced vasoconstriction can conceivably be seen as a stressor, thus triggering a regulatory mechanism through NO synthesis to bring the blood pressure back to normal levels. A release of vasopressin in a hemorrhagic shock model may show a more extended effect than seen in this work, because the vasoconstriction would be compensatory.

An alternative explanation for the loss of vasoconstriction is the degradation of vasopressin over time, either in the blood or before entering the bloodstream, although it has been shown in vitro not to degrade in rat serum for up to 8 hours [26]. The radioactive method used to measure vasopressin levels in this work does not distinguish between vasopressin and its degradation products or metabolites. A fraction of the measured plasma levels may conceivably correspond to degraded vasopressin or its metabolites that have not been filtered out by the kidneys. The plasma levels and calculated bioavailabilities in this work therefore indicate maximum values. A method such as high performance liquid chromatography coupled with mass spectroscopy (HPLC-MS) would be able to measure actual levels of intact vasopressin. It must be emphasized, however, that regardless of the actual bioavailability values, the measured effects on MAP are real and indicate sufficient levels of vasopressin to induce vasoconstriction. Release of vasopressin in the presence of a NO inhibitor such as L-NAME should tip the scale toward one of these hypotheses, depending on whether it prolongs vasoconstriction in healthy animals. A continuous infusion of vasopressin for 30 minutes or longer while monitoring MAP, as was done by Dworkin et al for the hepatic arterial bed, would also reveal whether the regulatory response is responsible for the observed behavior.

Another point to address if this device is to be used in a hemorrhagic shock model is that the peripheral circulation is already reduced due to reduced blood volume and vasoconstriction of the peripheral arterioles. Total peripheral vascular resistance is increased in this situation and may hamper the pharmacokinetics of vasopressin. The increasing plasma levels of vasopressin seen in this work despite vasoconstriction suggest that this may not be a problem, although the reduced blood volume may play a role in shock. This potential issue is only valid in a hemorrhagic shock model. Other conditions where peripheral circulation is unaffected would not encounter such issues.

This work did not address the issues of long-term implantation, where the formation of a fibrous capsule around the device may prevent diffusion of vasopressin. Work by Prescott et al has shown that this capsule does not affect the pharmacokinetics of leuprolide, another polypeptide with similar molecular weight to vasopressin[2]. Long-term implantation would also affect the stability of vasopressin in solution. A dual-chamber concept for the device is being investigated for that purpose, where the drug is kept in a lyophilized form in one chamber. The drug is rapidly reconstituted and released with water kept in a separate chamber upon activation.

Conclusions

The in vivo study presented in this work has provided proof of concept for the IRD^3 in an animal model. The lower bioavailability and magnitude of effects when compared to a subcutaneous injection are offset by better repeatability in the pharmacokinetic behavior and potential for remote or automatic delivery. Future development of the device may also increase bioavailability by reducing potential degradation mechanisms. The minimum efficacious dose of vasopressin has not been determined in hemorrhagic shock, and the ability of the device to address it with its current dose should be tested in a pathological model. Further research is required to address issues tied with long-term implantation. Applications for the device could extend beyond vasopressin to address acute conditions treatable with other drugs, such as atropine or epinephrine.

Authors' Contributions

HLHD contributed to the design and development of the project, the design, fabrication and characterization of the devices, the execution of all experiments and the writing of the manuscript. NE contributed to the design and development of the project, the design, fabrication and characterization of the devices and the writing of the manuscript. AMH contributed to the execution of the experiments and the writing of the manuscript. DJR contributed to the fabrication and characterization of the devices and the writing of the manuscript. MJC contributed to the design and development of the project and the writing of the manuscript.

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