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Robust, wireless gastric optogenetic implants for the study of peripheral pathways and applications in obesity*

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***Abstract*— Optogenetics has the potential to transform the study of organ functions in the peripheral nervous system via relatively easy access to the nerves and a direct link between the brain and organ systems. Implementation typically requires a static skeletal feature for the securement of a fiber. Unfortunately, the soft nature of peripheral nervous systems makes the wired fiber-optic approach less ideal for the study of the peripheral nervous system. Existing wireless approaches could bypass some constraints associated with optical fibers and thereby offer organ specificity. However, they suffer from durability loss due to considerable biological strains and unable to perform longitudinal experiments. Here, we propose a new class of wireless gastric optogenetic implant for identifying signaling pathways, in particular viscerosensory pathways, that can regulate food intake to treat obesity. Robust, wireless gastric optogenetic implants with a tubing-assisted U-shaped tether directly interface with nerve endings in the stomach with chronic stability in operation (> 100 kilocycles) and allows for optogenetic stimulations of vagus nerves in a freely behaving animal. We demonstrated utilities of the proposed wireless device in *in vivo* experiments. Results suggest the potential for identifying interventions for the treatment of obesity.**

***Clinical Relevance* — Identification of the roles of subpopulations in viscerosensory pathways would provide the platform for the development of better therapeutics for the treatment of obesity.**

I. Introduction

Optogenetics, one of the most powerful techniques, enables interrogation of neuronal function by targeted gene expression and activation of light-sensitive proteins with exceptional spatial and temporal resolution [1,2]. Implementation requires a remote light source and a light delivery scheme [3,4]. Optical fiber approaches have been instrumental in neuroscience experiments, however, the need for a static skeletal feature such as the skull limits its application to the study of brain dynamics [5]–[7]. That is, peripheral nervous systems (PNS) do not offer an environment where an optical fiber is secured to allow for uniform delivery of light to the targeted region. Consequently, this makes it less ideal for the study of peripheral pathways.

Modulation of organ systems and/or peripheral sensory pathways, in particular vagus nerves, has the potential for the development of better treatment for neurological disorders such as obesity via a direct link between the brain and organ systems. For example, the vagus is a logical place for interventions to treat obesity because it directly receives satiety information from the stomach and relays the information to the brain [8]. However, vagus nerves innervate many different internal organs, including the stomach, heart, lung, colon, and intestines [9], and it precludes non-specific approaches such as traditional electrical simulations. This justifies the use of a specific tool kit, optogenetics, for the study of the PNS.

Some approaches using advances in wireless technologies enabled wireless delivery of light to an organ system [10,11]. Although this wireless approach can bypass some constraints associated with wired fiber optics, it impedes the movement of an organ and/or suffers from durability loss due to an inability to secure a device to a targeted organ or nerves [12]. Devices that interface with nerves in the peripheral nervous system are subject to considerable biological strains associated with the natural movement of an animal. To maintain functionality over months for chronic applications requires a new strategy for device securement. Our lab recently developed a novel approach for high-throughput phenotyping of peripheral sensory pathways [13]. Results revealed that a pre-curved, sandwiched structure allows for operation with chronic stability up to a month.

Here, we propose a new approach using tubing to significantly increase the durability of a gastric device over months. The soft, fully implantable high-frequency range optoelectronic device with a tubing enhanced U-shaped tether directly interfaces with vagal afferent fibers in the stomach and delivers light to the nerve endings in a freely behaving animal with chronic stability in operation (> 100 kilocycles). When combined with advances in optogenetics technology, a gastric optogenetic implant with design features that mitigate inflammation and allow essentially permanent integration with the targeted nerves would provide us a unique set of tools that would lead to a greater understanding of neural circuits in controlling feeding, in particular viscerosensory pathways. We demonstrated the utilities of the device in *in vivo* experiments, and results revealed that optogenetic stimulations of vagal afferent fibers significantly suppress appetite. This suggests the potential for the widespread use of technology in neuroscience and for the development of better therapeutics for the treatment of obesity.

II. Methods

A. Procedures for Device Fabrication

We start with thin, flexible copper/polyimide bilayer (12

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μm / 18 μm thick) films attached to a glass slide. Next, we deposit photoresistor, and photo-lithography defines patterns on the film. It is followed by immersion in a developer solution and subsequent wet etching in the copper etchant for 30 secs. Rinse with distilled water yields contact pads and copper interconnections on a flexible substrate. We mount electronic components including microscale light-emitting diodes (μLEDs), transistors, and passive components on the substrate using a soldering machine. We use a soft polymer, Polydimethylsiloxane (PDMS), for encapsulations and a tubing for increasing durability. First, we insert a biocompatible tubing (inner diameter; 457 μm / outer diameter; 914 μm) through a thin, flexible tether and place it on top of μLED. Next, a simple coating process is applied to a thin, flexible tether first with a strain applied to form a U-shaped (Fig. 1(b)). Applications of a small amount of PDMS using a pipette with several attempts lead to a thin, U-shaped tubing enhanced tether. This process automatically fills inside of a tube with PDMS. Then, we cover the whole device with PDMS and they are cured at 80 ºC in a vacuum oven. Finally, these processes yield a thin, robust flexible wireless optoelectronic device with a U-shaped, tubing-assisted tether.

B. *Device Implantation*

All procedures to maintain and use mice were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. We used 2-4 months old male and female C57BL/6 mice. The mice were maintained on a 12-h:12-h light: dark cycle with ad libitum access to food and water. Mice received a bilateral nodose ganglion injection (left & right ganglion) of PHP.S-Ef1a-DIO-ChR2:YFP, and control mice received PHP.S-Ef1a-DIO-YFP virus injections. A soft wireless device was being implanted into the stomach. Our strategy to secure the soft ultra-thin, biocompatible optoelectronic device to the stomach is to 1) hold the tether with sharp-tipped forceps, 2) then puncture the stomach with the forceps. After recovery from surgical procedures, mice fasted overnight, and the following morning we photoactivated them upon granting access to standard chow for the duration of the 4-hour study; 1, 2, and 4-hour cumulative food intake were measured. We repeated the fast-refeed study (at least 5 days in between experiments) to examine different photoactivation frequencies (10 and 20 Hz; 10-ms pulse width).

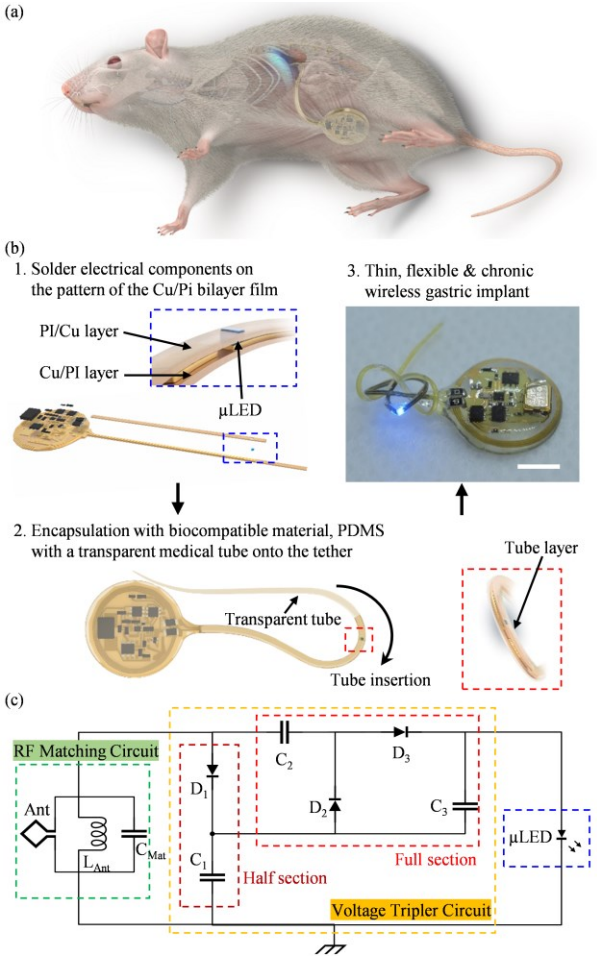

Figure 1. (a) Schematic illustration of a wireless gastric optogenetic implant. (b) Step by step procedures for device fabrication. (c) Circuit diagram of a gastric optogenetic implant.

III. RESULTS

An illustration shows the anatomy and the location of the device relative to the stomach (Fig. 1(a)). Because connective tissues quickly develop around it and there is not much movement in the abdomen, the wireless device does not need to be fixed in the abdomen. Fig. 1(b) illustrates step-by-step procedures for device fabrication, and Fig. 1(c) shows a circuit diagram of a gastric optogenetic implant. The wireless optoelectronic device consists of back-end electronics for energy harvesting and a neural interface for optogenetic stimulation of vagal afferents. The device harvests radio frequency energy at a frequency of 13.56 MHz from a remotely located wireless transmission antenna coil, converts voltages into currents, and routes currents to a light source, here μLED. The essential feature of the wireless device is a U-shaped, tubing-assisted tether that allows for intimate contact with nerve endings in the stomach with chronic stabilities in operation when implanted in a freely behaving animal.

To ensure the operational robustness of the proposed design, we performed cycling tests that investigate the reliability performance of the device in respect to strains. Results revealed that a U-shaped, tubing-assisted tether maintains functionalities at least over 100 kilocycles while a flexible structure without a tube stops working at a few kilocycles (Fig. 2(a)). We also assessed thermal characteristics

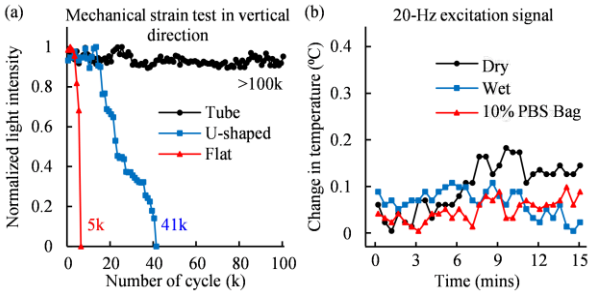

Figure 2. Characteristics of a gastric optogenetic implant. (a) Plot of light intensity Vs. number of cycles. (b) Thermal characteristics of a device in three different conditions: device is exposed to the air, immersed in saline solution, and placed onto 10 % PBS Bag, respectively.

of the device by using embedded temperature sensors in experiments where the light intensity of a device is monitored every 1k cycles under three different conditions (dry, wet, and 10% PBS solution). Measurement results showed that no detectable variation in temperature is observed during operation for 15 mins (Fig. 2(b)).

For the implanted device to be useful in *in vivo* experiments, the wireless power transmission (TX) system must enable robust activation of the device in a cage. The proposed wireless power TX system employs a triple-coil structure to achieve high efficiency in an open field arena for behavioral experiments. It offers broader, uniform wireless coverage at a given TX power, 2W, while electromagnetic fields in a reference structure (dual-coil antenna) are highly focused on regions, in particular near to coils (Fig.3) [14,15]. We also measured light intensity at 9 representative locations in an experimental assay (Fig. 4(a)). The results revealed the proposed triple coil antenna system can deliver enough light for activation of light-sensitive proteins at any locations of an assay and thereby enable robust activation of an implanted device throughout the volume of an assay (Fig. 4 (b) & (c)).

This indicates that our approach can provide more advantageous power budgets in a manner that activates an implanted device in a cage at low levels of TX power below 2 W. Although the high levels of TX power above 8 W could achieve a similar level of wireless coverage in a cage, corresponding increases in TX power may cause undesirable effects such as tissue damage associated with absorptions of transmitted radio frequency power [16]. When combined with a gastric wireless optogenetic implant, the proposed wireless

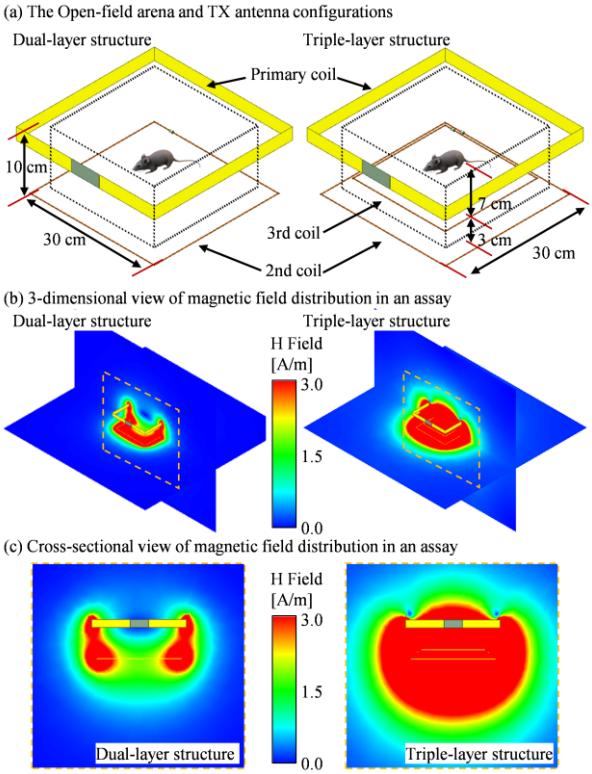

Figure 3. (a) Illustrations of an open field arena for behavioral modeling. Electromagnetic simulations in 3-dimensional view (b), and cross-sectional view (c): dual layer (left) & triple layer (right) coil antenna, respectively.

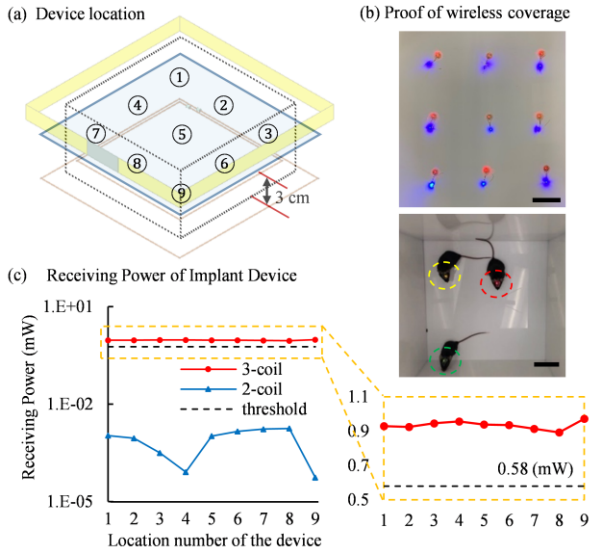

Figure 4. (a) Illustration of a setup for light intensity measurements (b) Images of devices (top) and animals with a device implanted (bottom); scale bar 5 cm. For the purposed of indication, we implanted a device into skin on top of a skull. (c) Plots of light intensity measurements at a height of 3 cm from the bottom of the cage. These exceed the threshold for activation of light sensitive proteins; an electrical power of 0.58 mW or optical intensity of 10.12 mW/mm^2.

TX system can yield powerful capabilities in the dissection of neural circuits and understanding of neuronal functions in a freely behaving animal.

To test the utilities of the proposed system in *in vivo* experiments, we performed sham studies first. Such experiments can determine whether implantation of the device does not affect the behavior of animals. Here, we monitored the food intake and the body weight of animals two weeks after implantation and sham surgeries, and results revealed that no significant difference between the two groups is observed (Fig. 5(a)). These studies justify feeding-related behavior experiments. Next, we performed behavior experiments: the fasting and refeeding test. To manipulate nerve endings in the stomach, light-sensitive proteins (ChR2) must be expressed to the targeted region.

Here, we use a viral infection approach, and an image confirms the expression of light-sensitive opsins in the nodose ganglions in Fig. 5(b). Fig. 5(c) shows the experiment protocol and an assay for behavior analysis. The implanted device directly interfaces with chemo-receptors innervating the corpus region of the stomach and delivers light for optogenetic stimulation of *Calca*+ vagal nerve endings in freely behaving animal (Fig. 5(d)) [17]. This indicates optogenetic stimulations of nerve endings in the stomach initiate a meal termination and send it to the brain, and therefore appetite is suppressed (Fig. 5(e)). These results suggest that the vagus nerve could serve as an intervention to regulate food intake for potentially treating obesity.

IV. DISCUSSIONS

Implanted devices maintain functionalities over a month and implantation did not cause any inflammation and lesions

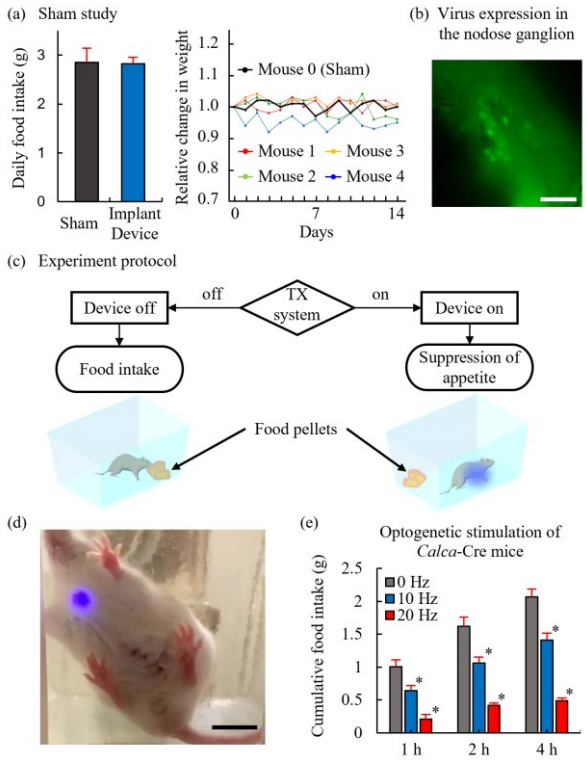

Figure 5. (a) Results of sham study (each group, n = 4) ($p = 0.71$). (b) Image of virus infection in nodose ganglions; scale bar 200 µm. (c) Illustration of experimental protocol. (d) Image of a mouse with a device implanted; Scale bar 1 cm. (e) Activation of CGRP neurons innervating in the stomach can significantly suppress appetite (n = 4). Bar graphs are mean ±SEM. Statistical comparisons were made using two-way repeated-measures ANOVA, Tukey's post hoc, which were two-tailed *t* test; $^{*}p < 0.001$.

to the stomach and abdomen cavity. This suggests our strategy to secure a tether to the stomach is successful and also proposes related opportunities for the use of the technology in neuroscience. A key to achieving enhanced durability is the use of tubing. Here, a thin, flexible transparent tubing with a diameter of 400 µm is inserted through a thin, flexible tether and placed onto µLED. After a simple coating process using a small pipette, the tubing paired with soft PDMS encapsulation layers would serve as an additional protective layer. Consequently, it would hold a µLED in place and ensure device operation. Mechanical failure mechanisms involve 1) disconnection between a contact pad on a flexible substrate and a µLED and 2) cracks on encapsulating materials. These are linked to biological strains and/or mechanical stress due to natural motions of an animal (contortion of abdomen cavity, gastric distension) [11], [18,19]. When combined with a U-shaped structure, the tubing-assisted tether would significantly reduce mechanical stress, thereby increase durability, and therefore ensure chronic stabilities in operation (> 100 kilocycles).

We could also optimize the degree of bending, the ratios of candidate acid-resistant polymers to a curing agent, and the length of a tube to extend the lifetime of a device over two months. These three are factors that determine device lifetime. Depending on target organs, in particular shape and surface of a target organ, we could determine a bending curvature of a tether, the degree of stiffness of encapsulation layers, and the length of a tube. For example, the stomach has a relatively flat surface compared with the intestines. Devices can be customized to the stomach with design features, low curvature (< 3 mm), and length of a tube (4 mm) while they can be tailed to the intestine with design features, high curvature (> 6 mm), and length of a tube (10 mm).

An implanted device may not receive enough power due to misalignment and the increased gap between a device and the TX coil antenna when an animal stands on its hind legs or leans against a wall of an experimental assay. This could be problematic. The proposed triple-coil antenna with metal strips can enable robust activation throughout the volume of an assay at a transmitted power level of 2 W. Placement of an additional coil antenna at a height of 5 cm from the ground plane and paired with two bottom coil antennas offers uniform coverage of wireless power. The gaps between coil antennas could be adjusted to enable high or low profile power distributions.

Advanced antenna designs with radio frequency control schemes can further increase the wireless coverage in a cage at a lower level of TX power below 2 W. For example, a diagonal triple-loop design can allow coverage even with significant tilted angles from the in-plane orientation. Multi-diagonal layouts can further enhance the coverage and improve the uniformity of wireless transmittance over the bottom of the cage and across large areas. When combined with motion tracking algorithms that allow for selective/adaptive control of multiple coils, advanced antenna coil systems can significantly expand wireless coverage and eliminate residual dependence of transmitted power on orientation angles between the TX system and the implantable device. This approach represents an attractive solution to certain classes of neuroscience experiments, such as studies of complex social behavior within a group of animals, which require an experimental assay with dimensions of 60 cm by 60 cm and demand extensive coverage. High levels of transmitted power above 8 W could achieve similar levels of coverage, however, the corresponding increase in TX power may exceed maximum exposure limits suggested by IEEE or FCC [20,21].

Unexpected research findings include the identification of a putative chemosensory pathway in the stomach for controlling appetite. The latter result is surprising, given the dogma that mechanosensory signals arising from the stomach, rather than chemosensation, contributes to appetite suppression. This suggests that activation of nerve endings in the mucosa can suppress appetite in a different manner that gastric distension initiates meal termination. The next logical steps would be to identify functional roles of sub-cell types (Ucn & Sst) innervating in the stomach and/or intestines. In addition, straightforward extension of the proposed technology includes experiments with large animals such as primates. Due to large dimensions of an organ in primates, limitations on device form factors would be lifted. In this scenario, we envision an implantable device with a coil (dimensions of 3 cm by 3 cm) embedded harvests radio frequency energy from a smartphone via near-field communication.

V. Conclusion

We demonstrated that the proposed device is capable of an intimate electrophysiological interface via a thin, U-shaped

tubing-assisted tether. These innovative technologies allow experiments that examine subtypes suppressing feeding, and successful results will provide a foundational understanding of how the complex feeding loop, in particular downstream pathways including the gut → vagus nerves → NTS, works to regulate energy homeostasis. First, the dissemination of vagus nodose will allow us to link the genetic identity of vagal afferent subpopulations with specific functions. It has been found that the vagus nerve is involved in decreasing food intake [22]. However, a recent study reveals that the vagus nerve may be involved in increasing food intake [23]. This suggests that subpopulations of gastric vagal afferent neurons differentially control feeding behavior and affect. Therefore, with our approach, we can determine the role of gastric vagal afferent subpopulations in controlling appetite. This will address two critical questions: 1) the general importance of ascending sensory signals from the stomach for satiation and satiety maintenance and 2) the relative physiological contributions of vagal sensory signals to satiation and satiety. Moreover, it will be important to determine whether appetite suppression induced by gastric vagal afferent activation is attenuated in obese mice and whether chronic activation of vagal afferent endings in the stomach can reverse obesity. Identification of viscerosensory pathways that suppress appetite will have direct clinical importance for potentially developing novel therapeutic targets for treating obesity.

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