FULLY IMPLANTABLE WIRELESS AND BATTERY-FREE ORGAN INTERFACES

by

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I would like to dedicate this work to my parents
Richard Burton and Natsuko Burton
for their love and support.
You have always been there for me.

I would also like to dedicate this work
to the rest of my family Hannah, Nicole, and Nicolas
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ABSTRACT

Seamless organ interfaces combined with high fidelity readouts and modulation capabilities offer unparalleled insights into the central and peripheral nervous system and musculo-skeletal system. A new class of wireless, battery-free platforms enable long-term experiments with continuous uninterrupted recording and stimulation, with capabilities that match or exceed those of current wired or battery-powered platforms. Combined with soft and flexible mechanics, these devices are fully implantable in small animal models enabling studies without impacting the subject’s behavior or mobility, with fast recovery times post-surgery, with reduced infection risks, and operate with lifetimes that exceed those of the test subjects. Development of this new class of device is critical to bridge the gap between preclinical and clinical research to enable new diagnostic tools to dissect understudied organ systems and to further the development of therapeutic tools for motor disorders and spinal cord injuries.

Specifically, I have expanded these platforms with antenna designs optimized for use in highly miniaturized form factor to facilitate subdermal implantation in freely moving young mice. This allows long-term experiments to study complex behavioral circuits by recording cell-specific neural dynamics¹. I have also implemented communication protocols that allow existing systems to receive programmed stimulation parameters without requiring additional circuity or power. This allows miniature systems to be subdermally implanted in rats to electrically modulating cell specific neural pathways, in the deep brain using surface engineered microelectrodes to study stimulation dosing and their use in therapeutic treatment of motor disorders². I also extended the technological platform for osseosurface electronics to allow for two-way communication for devices with multimodal capabilities of stimulation and recording. This enabled devices to capture long-term metrics of bone health in real time, paving the way for personalized treatment of the
musculoskeletal system\textsuperscript{3}. Combined, I have advanced wireless battery-free platforms to effectively study targeted organs in freely moving subjects through the optimization of antenna and system designs, device mechanics and flexible electronics fabrication schemes and implementation of communication protocols. These systems have also led to the expansion of current experimental paradigms to study freely behaving subjects which can provide significant insight into functional mechanisms of the nervous system and musculoskeletal system which are currently substantially limited by conventional tethered, and battery powered approaches.
1. INTRODUCTION: FULLY IMPLANTABLE, WIRELESS, BATTERY-FREE SOFT ORGAN INTERFACES

Recent advances in organ interfaces have quantitatively expanded current paradigms for exploratory research and development of diagnostics and therapeutics system. The rapid development of these interfaces are realized through effective combination of advances in electronics, materials, and sensors that have improved operation of devices to sense and stimulate to further our understanding of fundamental mechanisms in the nervous and musculoskeletal system.

The nervous system controls all regions of anatomy through a complex system of interconnected neural pathways using chemical transduction and electrical propagation between interconnected axons, dendrites, and synapses to control behaviors, sense stimuli, and regulate organ functions. Investigation of these neural circuits are crucial in understanding neuropsychiatric disorders that affect over one in five adults and is the leading cause of disability in the U.S. with over 18% of average life lost due to disability and premature mortality4,5. Current modalities to interface with the central nervous system include electrical, optical, and chemical sensors that have contributed to knowledge of the functional mechanisms of the brain6–8. However, these systems are limited in their ability to effectively leverage sensing modalities to enable continuous chronic experiments in freely behaving rodents which are critical for mechanistic studies and development of therapeutic approaches. To develop effective tools in detecting neurological disorders, it is important to improve technologies that expand current experimental paradigms to understand long term circuit level functions during specific behavior such as depression and reward pathways that allow us to monitor development of neuropsychiatric disorders and development of cognition and learning on a cell specific level.
Neural recording systems are important to detect circuit level activity, however it is also important to develop systems for neuromodulation for therapeutic treatment of neurological diseases. For example, movement disorders in patients with Alzheimer’s and Parkinson’s and traumatic injuries such as spinal cord injuries (SCI). This affects 500,000 people every year with a risk of developing secondary conditions that can be debilitating and even life-threatening due to inadequate medical care and rehabilitation services\(^9\). Current technologies for neural modulation are limited by tethered approaches for long term stimulation that limit subject mobility and behaviors which can impact experimental outcome. Reducing overall impact on the animal’s behavior and mobility is important when investigating the functional circuits of the brain. Furthermore, technologies that enable continuous, long-term, wireless operation can significantly improve systematic insight on dosing of stimulation parameters for effective therapeutic treatments.

Another organ system of interest is the musculoskeletal system which is understudied due to the lack of devices that are capable of interfacing with the bone for long periods of time without delamination from the bone surface. The bone provides as stable mounting location that allows for permanent bonding of bio-interfaces that allow for a rugged sensor to measure quality of bone health and stimulation for therapeutic treatment. Monitoring bone health is important in patients with osteoporosis and osteopenia due to the increased risk in developing fragility fractures that accounts for more hospital bed-days than myocardial infarction or prostate cancer\(^{10}\) and will cost over $25 billion per year by 2025\(^{11}\). It is therefore important to develop technologies that can continuously monitor long term bone quality\(^{12}\) and enable a point-of-care platform to improve quality of life for patients.
Current technologies that interface with the nervous and musculoskeletal system often utilize optical fibers or wired tethers that can connect to existing hardware for sensing and stimulation. However, these interfere with the animal’s mobility and behavior while confining experimental paradigms to studying single subjects in open arenas. Tethered approaches increase risk of infection, animal entanglement, and introduce motion artifacts due to micromotions of the interface that can lead to scarring of surrounding tissues. To solve this issue, wireless, battery-powered systems have been utilized to study multiple subjects. However, these systems require large and bulky battery packs which can impact animal mobility while suffering from limited operation times thus requiring continuous device monitoring and recharging which can impact the subject’s natural behaviors.

The use of wireless, battery-free systems expands current sensing and stimulation capabilities without drawbacks set by tethered and battery-powered systems. However, the current state of these platforms requires the use of passive systems to stimulate or record bio signals using application-specific integrated circuits (ASIC) which have a high cost of entry. To solve this issue, I have advanced current state of wireless battery-free systems. Here I have advanced power harvesting and communication through optimization of antenna designs combined with off-the-shelf components for scalable manufacturing to enable board dissemination of these devices for use in small animal research. These devices are capable of both wireless power harvesting and communication allowing for multimodal platforms for both to stimulate and record during long-term uninterrupted experiments to provide useful insight in understudied areas of the body.

The evolution of low-cost manufacturable flexible circuits has allowed for devices to transition away from rigid board design allowing for fully implantable systems that conform to the target organ. Here I have advanced biocompatible encapsulations, design schemes, and flexible
device mechanics to seamlessly integrate with the targeted organ with minimal impact on surrounding tissues while extending device lifetimes that can surpass that of the subject. The fully implantable miniature device designs leverage the use of small animal subjects such as mice and rats as a translational model to drive studies and technologies towards clinical research as shown in Fig. 1. Small animal subjects open possibilities for genetic control over specific traits and diseases of interest that can be studied in large populations. These animal models also offer a wide range of cell specific targeting using commercially available viral targeting agents (blue) that allow for both the recording and modulation of neural activity (orange). They also provide useful insights towards testing dosing parameters for electrotherapy in the deep brain and spinal cord and prove a useful model to study new materials to enable greater biocompatibility and flexibility to improve long-term adhesion of organ interfaces (green). This dissertation presents solutions to longstanding problems in organ interfaces by solving limitations of current power supply, communication, and bio-interface to enabling greater understanding of fundamental mechanisms and lay the technological groundwork for the development of new therapeutic and diagnostic tools that directly treat patients.

*Figure 1. Overview of technologies and genetic tools that interface with various organ systems to dissect and treat neurological diseases and motor injuries*. Adapted/Translated by permission from Springer Nature Customer Service Centre GmbH:
1.1 Wireless Battery-Free Technologies

Current technologies to interface with soft tissues include physical tethers such as wires or optical fibers that allow for connectivity to external supporting hardware or wireless battery powered systems. However, these technologies limit experimental paradigms and animal models that can be studied effectively without impacting their mobility or behavior. Wireless battery-free platforms avoid drawbacks of current tethered and battery powered systems where animals and devices need to be constantly monitored to prevent entanglement that induce motion artifacts during recording and to ensure that devices are always charged. A wireless, battery-free device platform enables expansion towards long-term experiments to study freely moving, socially interacting subject in ethologically relevant environments.

Creation of these wireless battery-free devices require careful deliberation between formfactor, power harvesting, and communication requirements that are tailored towards specific animal models and application. These devices play a key role in modern exploratory research, development of therapeutic treatments, and provide opportunities to develop point-of-care applications. The transition from a battery powered to a battery-free system also requires attention to ensure continuous power delivery while ensuring minimal device heating from excess harvested power as it does not rely on batteries to store charge. Here I will discuss current state of wireless battery-free technologies that communicate and harvest power using concepts of photovoltaic (PV), ultrasound (US), near field, and far field systems as shown in Fig. 2a-d. These modalities prove effective in battery powered systems as well as designs that operate with ASIC. However, scalable manufacturing of wireless, battery-free systems requires off-the shelf components which
involve additional design requirement to device dimensions, heat dissipation, power transfer, and communication. Leveraging WPT and communication strategies in a battery-free platform will expand current testing paradigms capable of studying long term, uninterrupted experiments of freely behaving subjects to allow for greater insight in understudied areas such as the nervous and musculoskeletal system.

Figure 2. Methods for wireless power transfer and wireless communication using directed energy sources. a Operating mechanism of photovoltaic power transfer (top); Strategy for optical communication (bottom)\textsuperscript{15}. © December 2018 IEEE. b Operating mechanism of ultrasound power transfer using a piezo-electric crystal (top); Triboelectric membrane used for power transfer (bottom)\textsuperscript{15}. Reprinted with permission from AAAS. c Operating mechanism of a magnetic resonant coupling within the near field regime (top); Rendering of E-textile for magnetic resonance power transfer to drive large body sensor networks (bottom)\textsuperscript{16}. d Operating mechanism of far field RF for power transfer (top); rendering and simulation of electric field distribution using meta-material textile to enhance communication for body networks (bottom)\textsuperscript{17}. Adapted/Translated by permission from Springer Nature Customer Service Centre GmbH: Springer Nature Electronics, Tian, X., Lee, P.M., Tan, Y.J. et al., Wireless body sensor networks based on metamaterial textiles, June 17, 2019.

1.1.1 Wireless Power Transfer

Devices utilizing the PV effect are capable of harvesting power from the near infrared 780–850 nm\textsuperscript{18,19} and visible spectrum ~400–750 nm\textsuperscript{20} as shown in Fig. 2a. Many of these applications harvest energy from sun light, however in application where continuous delivery of light is needed,
a directed light source has been shown to enable device operation in implantable applications. These PV diodes are highly miniaturized with an area of 250 µm × 57 µm\(^1\), however, require ASIC for their miniature designs and low power consumption. The ASIC fabrication has a high entry-barrier in terms of cost and requiring liaising with semiconductor manufactures making this technology suited for only high-volume production. PV devices are also limited in their application to interface with deep tissues due to absorption and scattering light which can heat up tissues showing operations using directed light source operate up to 20 minutes when continuously powered (250-300 mW/mm\(^2\)) before tissue heat up by 5 °C\(^2\).

US is another viable technology for WPT allowing for the propagation of mechanical energy through soft tissues >10 cm as shown in Fig. 2b\(^2\). This is because of the longer wavelength \(\sim 0.1–1.5\) mm that can penetrate deep tissues\(^3\). To convert the mechanical energy into electrical energy, piezoelectric or triboelectric systems are used to convert the mechanical energy into electrical energy. Piezoelectric systems is shown to have capabilities to generate 80 mW of power at depth of 5 cm which attributed to the maximum of 720 mW/cm\(^2\) of US power capable of being delivered compared to 10 mW/cm\(^2\) with radio waves\(^4\)-\(^6\). However, in battery-free application, excess power can cause heating of surrounding tissue. This requires devices to be optimized to reduce power consumption to levels (100 µW) which are sufficient to continuously record electromyogram and electroneurogram in anesthetized rats\(^6\). US also requires direct coupling with the body and alignment with the implanted device thus limiting its application in freely moving subjects.

Magnetic resonant coupling (MRC) operates in the non-radiative near field regime and is normally used within distances between 0 and \(\lambda\) from the transmitter with effective power casting distances up to 60 cm as shown in Fig. 2c\(^2\). Frequencies between 0.1 and 200 MHz are utilized.
for near field WPT with most systems operating at 13.56 MHz which is reserved internationally for medical and scientific purposes. Combined with low absorption rate $< 20\text{mW kg}^{-1}$ between an external primary and secondary antenna allows for implantation of device in deep tissues which is important for translation of these devices toward clinical models. MRC relies on antenna matching of the primary and secondary antennas where the primary antenna generates alternating current that generates an electromagnetic field that induce current on the secondary antenna, which is subsequently rectified and regulated to allow for stable operation of the device. When translating towards larger subjects and humans, WPT efficiencies of MRC are often inadequate as operational distances are less than the diameter of the transmitting coil antenna with a maximum distance of 1 m. In these cases, implantable devices can be paired with wearable systems that allow for directed MRC power delivery as shown in Fig. 2c (bottom).

Devices that utilize far field WPT usually operate at distances above $2\lambda$ with operational ranges spanning from 0.1 m and 3 m as shown in Fig. 2d. Operation frequencies for these systems can range from 200 MHz to 2.5 GHz with similar absorption rate by the body ($\sim 0.1–6.15\text{ W/kg}$) as near field WPT$^{17,28}$. Advantages of this system is its capability to transmit power at large distances thus allowing for far field WPT schemes to be effective in the use in wireless battery free devices in the translation of devices towards use in humans. On the other hand, power efficiencies at these distances limit their capabilities for stimulation and recording and often require the use of custom ASIC. Devices also require larger antennas for greater power efficiencies that are susceptible to being detuned with biological tissue and the surrounding environment.

Recent development of advanced integrated circuits (IC) specifically designed to harvest energies from electromagnetic fields has enabled a majority of wireless battery-free systems to operate using near and far field power transfer$^6$. However, far field antennas are affected by lossy
dielectric environments such as the body. Therefore, near field WPT is optimal to enable long-term uninterrupted operation of battery-free systems that are subdermally implanted in small animal subjects and operated in defined environments.

Current antenna designs have not yet been optimized for power harvesting for subdermally implantation in small animal subjects such as adolescent mice to study long-term neural activation through photometric recording. Here devices must fit within the area above the skull (<11 mm × <8 mm), which limits capable power delivery. The antennas must also be optimized to ensure stable operating voltages with enough power to ensure continuous operation of the device. The turns, spacing, and trace width of the device antenna must also be optimized to ensure power harvesting is greater than power consumption of the device (>9 mW) throughout the volume a mice home cage (>18 cm x >12 cm). An additional factor of safety for power harvesting (150%) is also required to allow continuous operation during animal postures of rearing and walking which lead to angular misalignments which reduces WPT efficiencies.

When translating devices for use in larger subjects such as rats for electrical stimulation, devices must operate in a significantly larger home cage design (>28 cm x >22 cm) than mice. WPT must allow for power harvesting >10 mW to match the increased power consumption due to the increase in voltage requirements for compliant electrical stimulation. Devices that electrically stimulate required higher voltage compliance up to 5 V to enable a wide range for deep brain stimulation to accommodate a variety of electrodes. In case of micro sized electrodes voltage compliances can become relatively high. This requires antenna designed for optimal power harvesting to greater than 5 V within the volume of the cage while accounting for angular misalignment of the device. Due to the high voltage compliance, devices must be monitored to
ensure minimal heat generation to minimize detrimental effect on surrounding tissues and must meet safety standards (< 1 °C) of the American Association of Medical Instrumentation.

Finally, multimodal devices require greater power harvesting capabilities (> 11 mW) to ensure control of both stimulation, recording and computation of high-fidelity physiological. The Improvements in the WPT demonstrated in this work has enabled creation of new digitally controllable, wireless, battery-free systems that allows for expansion of current experimental paradigms to study freely moving subjects in ethologically relevant environments over long time periods, which cannot easily be achieved with current battery powered or tethered devices. Optimizing WPT systems for use in small animal subjects provides opportunities for future devices platforms to extend current stimulation and sensing capabilities that can improve mechanistic insight to understudied regions of the body.

1.1.2 Wireless Communication Schemes

PV power transfer offers methods for direct integration of wireless communication with low impact on power consumption (< 0.5 mA) in miniaturized form factors using ASIC designs (250 µm x 57 µm) or using off-the-shelf components (> 1.0 x 0.5 mm) that communicate by modulating the light intensity as shown in Fig. 2a. These devices optically communicate by digitally transmitting data packets to minimize cross talk between multiple devices or pulse-position modulation which converts direct analog signals into time-based pulses for use in energy efficient applications. To improve depth of communication with implanted devices, IR wavelengths (940 nm - 950 nm) are utilize to reduce the scattering coefficient to around 1 mm\(^{-1}\) allowing for transmission through thicker layers of tissue. However, optical forms of communication are still limited in their applications for use in subdermally implanted devices in
deep tissues (< 3 mm) while also requiring direct line of sight with the receiver (1 m – 10 m). Here optical methods for communication have been shown to be effective in small animal subjects allowing for real time recording of neural activation through photometric recoding of calcium dynamics with data transmission speeds faster than the rise time of GCaMP (> 20 Hz)\textsuperscript{31}.

US communication allow for transmission of data through deep tissues of < 5 cm by using an ON-OFF keying modulation with a transmission voltage of 10 Vp-p as shown in Fig. 2b\textsuperscript{32}. US communication is often used in applications that require lower power consumption and smaller device size (750 µm x 750 µm), US backscattering processes is used by modulating the ultrasound carrier signals (0.5 MHz – 5 MHz) with the electrical impedance of the implantable transducer\textsuperscript{26}. These systems are highly miniaturized which allow implantation using a syringe, however, require the use of ASIC designs\textsuperscript{14}. A major drawback of this method of communication is that it is difficult to implement in freely moving animal subjects as the mechanical coupling between the transmitter and receiver can easily be disrupted by air gaps thus requiring US gel to couple the transmitter and the skin. Neuromodulation technologies that utilize US communication enable communication with multiple devices and individual control through digital protocols with speeds up to 11 kbps. Here, US communication has been used to stimulate the sciatic nerve under thick muscle layers (< 5 cm) to generate muscle action potentials and compound action potentials \textsuperscript{33,34}. However, the need to acoustically couple the transducer impacts the animal mobility limiting operations to short-term experiments.

Most near field communication (NFC) technologies and protocols are mature allowing for low-cost integration with security features to protect sensitive medical information and anti-collision features to enable reading of multiple devices as shown in Fig. 2c. NFC operates in the frequency band of 13.56 MHz and communicates to the implanted device by modulating power,
while the implanted device transmits information using load modulation\textsuperscript{6,35}. However, NFC systems are limited in communication distance to up to 1 m over air and implantation depths of less than 8 cm making it difficult for continuous data transfer in device translation towards large animal subjects and humans without providing a reader scheme that is located in proximity. In applications where two way communication is needed, specialized NFC ICs are widely available in small packages (2.5 mm x 2.5 mm) offering speeds up to 420 kbps and ability to simultaneously harvest power and regulate voltages\textsuperscript{36}. The addition of extra components, however, limits the location of subdermal implantation to large open spaces such as the dorsal region or subdermal implantation on the skull. Devices that locate the thin communication, computation and control device and provide a soft interconnect to the bio-interface have been demonstrated. For one way communication, NFC has shown its effectiveness by allowing for subdermal implantation (< 1 mm) above the skull without requiring additional components while allowing for real time control over stimulation parameters for deep brain stimulation\textsuperscript{37,38}. For two-way communication, NFC has been shown to be useful for the transmission of multiple bio-signals with high fidelity and speed (14 bit, 2 kHz)\textsuperscript{39}.

Far field communication operates in an ultra-high frequency (UHF) band with common frequencies of 433 MHz or 915 MHz and allows for long distance communication that can extend up to 500 m in air and allow for high-speed communication often using backscatter techniques to achieve speeds of 5 Mbps with very little power consumption (6 mW) as shown in Fig. 2d. However, when implanted, communication distances are only capable of transmitting though < 8 cm of tissue. Many of these systems also require ASIC designs\textsuperscript{6} and requires careful attention to detuning of the antenna with the surrounding tissues. This limits far field communications for use in implantable systems and often are used in wearable systems minimizing risk of antenna
detuning. These systems are useful in applications that require high speed communication of multiple channels which has been used to record and stimulate neural activity of flying subjects in large areas > 2 m from a communication station\(^{40}\) where communication systems are located outside the subject and bio-interfaces are realized with transcutaneous wires.

Communication methods (summarized in Table 2) enable effective wireless battery-free systems to achieve goals for stable data transmission in freely moving subjects. Compared to analog systems. Digitally controlled devices present greater utility by allow precise control for real-time stimulation and recording of high-fidelity bio-signals with multiple devices without crosstalk. Communication modalities and mediums have advantages and drawbacks and need to be carefully selected to match target applications.

**Table 1. Summary of communication methods used in wireless battery free devices.**

<table>
<thead>
<tr>
<th>Modality</th>
<th>Transmission</th>
<th>Comm. Distance</th>
<th>Implant Depth</th>
<th>Data Rate</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photovoltaic</td>
<td>Infrared light (940–950 nm)(^{41})</td>
<td>1-10 m(^{4})</td>
<td>&lt; 3 mm(^{41})</td>
<td>500 bps</td>
<td>&gt;250 (\mu)m x 57 (\mu)m(^{14})</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Ultrasound backscatter (0.5-5MHz)(^{42})</td>
<td>Direct contact with body(^{15,43})</td>
<td>&lt; 5 cm(^{15,43})</td>
<td>10 kbps(^{42})</td>
<td>&gt;750 (\mu)m x 750 (\mu)m(^{42})</td>
</tr>
<tr>
<td>Near field Communication</td>
<td>Backscatter 13.56 MH(^{44})</td>
<td>&lt; 1 m(^{44})</td>
<td>&lt; 8 cm(^{45})</td>
<td>420 kbps(^{36})</td>
<td>2.5 mm x 2.5 mm(^{13})</td>
</tr>
<tr>
<td>Far field Communication</td>
<td>RF Radiation 0.5-2.4 GHz(^{36})</td>
<td>1-500 m(^{40,46})</td>
<td>&lt; 8 cm(^{42})</td>
<td>0.01-5 Mbps(^{48})</td>
<td>2.36 x 1.88 mm(^{40})</td>
</tr>
</tbody>
</table>

**1.2 Designs for Fully Implantable Devices**

Device mechanics and materials are critical in the development of fully implantable devices that are composed of hard silicon chipsets to reduce their damage to surrounding soft tissues which will improve operational lifetimes, surgical procedure, and interface performance\(^{7,49}\). Combined with WPT a new class of fully implantable, wireless, battery-free systems are realized allowing for long term uninterrupted operation of devices without limits set by tethered or battery powered devices with capabilities for high fidelity readouts of bio-signals and control over
stimulus parameters in small animal subjects while expanding current experimental paradigms for translational use towards clinical model. Examples of mechanical designs that are utilized in implantable systems include the use of strain isolated interconnects and thin film designs to interface with soft neurological tissues while material design include surface engineered electrode interfaces and the use of soft biocompatible materials for device encapsulation such as hydrogels and silicones as highlighted in Fig. 3a-d.

Figure 3. Soft flexible materials for improving biocompatibility of implanted systems. a Structural design to improve mechanical matching with soft tissues. Adapted/Translated by permission from Springer Nature Customer Service Centre GmbH: Springer Nature Materials, Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics, Dae-Hyeong Kim et al, April 18, 2010. b Utilizing serpentine structures to reduce strain between the device and organ interface. c Hydrogel structures to improve interfacing with the tissue. © December 2013 IEEE, reuse with permission from Frontier Neuroengineering. d Soft neural interface based on silicone materials. Reprinted with permission from AAAS.
1.2.1 Device Mechanics

In thin film mechanics the thickness of the material scales linearly for bending strain and cubically with stiffness thus making thin film designs critical when incorporating semiconductors and metals in designs that require a conformal interface with the target organ. Here Fig. 3a shows a conformal neural interface with 30 electrodes in a thin mesh structure that allows for high-fidelity recording and stimulation of the central nervous system with low spatial deviation of electrode placements\textsuperscript{54}. These structural designs enable the use of soft interfaces that can conform to irregular curved surfaces and depth electrodes to have intimate interaction with the central and peripheral nervous system\textsuperscript{54}. However, these thinner designs require expensive manufacturing process in clean room environments and require attention for adequate layer adhesion and careful preparation of these thin metal layers by pre-straining the polymer substrate or develop engineered microcrack patterns in the substrate to enable a functionally stretchable interface\textsuperscript{55,56}.

By incorporating devices with strain absorbing configurations using serpentine structures and meshes allows for out-of-plane deformations, which exhibits a modulus closer to that of soft tissues while accommodating for high tensile strains as shown in Fig. 3b\textsuperscript{57} with robust mechanics that reduce risks of microcracking. This serpentine scheme is often utilized in highly mobile areas of the body in both the interface and implanted device which requires more than 100,000 cycles of repetitive stretching and compression with < 8% of strain to prevent plastic deformation and peak as well as to facilitate probe placement during surgery with peak deformations of 150\textsuperscript{38}. When developing devices for low-cost scalable manufacturing, monolithic designs are used with rigid islands formed with densely packed ICs with serpentine interconnects between islands that can withstand strains well beyond capable range of biological motions while enabling a fully implantable system that can last the lifetime of the subject. However, scalable designs that utilize
flexible printed circuit board manufacturing are not biocompatible and require additional encapsulation methods to prevent water ingress into sensitive electronics and to match the modulus of soft tissues to prevent tissue scarring.

1.2.2 Device Materials and Encapsulation

While mechanical designs increase the softness of devices, the overall characteristics of the semiconductor and metals are harder than the surrounding tissues. Advances in biomaterial research has shown capabilities for ultra-soft biocompatible materials such as conductive hydrogels which have been shown to have equal or lower modulus as the surrounding tissue, thus reducing scarring and quality of electrically interfacing with neurons\textsuperscript{58,59}. These hydrogels can be integrated with dissolvable scaffolding to allow for synaptic growth towards the electrodes and decrease in charge injection capacity\textsuperscript{60,61}. However, these solutions require combined efforts in both material and tissue engineering to allow for such a scaffold gel structure to be fully utilized. Hydrogel materials are also difficult to manufacture for large scale dissemination and require special attention to the storage environments of these devices\textsuperscript{62}.

More scalable techniques and platinum cured silicone materials can be used to produce similar effects of hydrogels in matching the device and tissue modulus to minimize. Silicones are highly adaptable and depending on the polymer-precursors, cross-linkers, and curing environment allowing for a range of modulus of elasticities to interface with various target organs such as the brain, spinal cord, and heart\textsuperscript{7}. PDMS is a specific form of silicon that is widely utilized in implantable devices as it is highly flexible, chemically inert, and doesn’t absorb water as shown in Fig. 3d\textsuperscript{63}. By integrating thin film mechanics and PDMS a soft interface between the target organ and device can be realized allowing for long-term implantation with minimal scarring of
surrounding tissues. However, PDMS is permeable to water, which can overtime damage complex electrical circuits underneath. Inorganic coating methods like SiO2 and Al2O3 are widely used to encapsulate implanted devices however these methods require extreme environments with high temperatures above 300°C that can damage electronics, therefore polymers like Parylene and polyurethane are widely combined with the use of silicones to act as a water barrier due to their low permeability to water while matching mechanical modulus between the device and tissue\textsuperscript{64,65}. It is important to utilize the correct mechanical and material design in specific application to target specific organs to allow for long-term implantability of devices with minimal impact on the subject’s mobility and behavior, while minimizing immunological reaction by matching the modulus between material and tissue and utilizing chemically inert materials for biocompatibility.

1.3 Sensing/Stimulation Probe Design

Wireless battery-free platforms offer favorable mechanics to both sensing and simulation modalities to intimately integrate with the target organ to yield high fidelity recordings and effective stimulation capabilities that outperform their tethered counterparts. The following section will summarize common modalities to interface with the body and current techniques utilized to improve current wired modalities to enable low power and miniature interfaces that can be incorporated in fully implantable wireless battery-free platforms. The modalities include chemical, thermal, optical, pharmaceutical, electrical, and mechanical concepts each with specific applications as shown in Fig. 4a-f.
1.3.1 Direct Electrical Recording and Stimulation

Electrical techniques to interface with the central and peripheral nervous system are well established and are used in clinical applications such as deep brain stimulation, spinal cord stimulation, and electroencephalography as shown in Fig. 4a. Current electrode designs leverage noble metals such as platinum and gold that allow for biocompatibility and high resistance to corrosion from bio-fluids. However for electrodes that require precise targeting, micro electrodes (<100 µm, diameter) are required with additional surface engineered designs such as ceramics using iridium oxide or conductive polymers such as poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) to expand capabilities of sensing and stimulation without exceeding...
charge injection capacity that can lead to damage of the electrode and surrounding tissue\textsuperscript{70–72}. Implementing biphasic stimulation with a reversed polarity pulse after the leading stimulation pulse counteracts the electrochemical processes at the electrode surface. This balances charges between the stimulation electrodes thus improving electrode lifetimes and minimizes changes in pH and dissolution of metals in surrounding tissues\textsuperscript{73}. Biphasic pulses also enables use of lower stimulation currents compared to a monophasic pulse to achieve the same neural activity with improvement up to 50\% for the activation of resting neurons\textsuperscript{74}. Electrical modulation of the nervous and musculoskeletal system is accomplished by generating potential gradients within the tissue to excite surrounding neurons which can be adjusted by controlling stimulation parameters such as amplitude, frequency, pulse width, and polarity to enable adjustments to the rate of excitation of the nerves\textsuperscript{75}.

Direct electrical recording measures average neuronal excitation which generates an electric potential that are measured across a pair of electrodes. The mode of operation therefore limits electrical techniques to distinguish between cell types which can be difficult in application that requiring understanding of complex behavioral circuits that are comprised of multiple overlapping projections with assorted cell types for excitation and inhibition\textsuperscript{13}. In cases where specific projects and cell are studied, pharmaceutical interventions are often utilized to study specific circuits of interest while using electrical methods for sensing and stimulation\textsuperscript{76}. These fundamental constraints of electrical techniques limit their ability to effectivity understand specific neural circuit and deliver controlled therapeutic outcomes.
1.3.2 Cell-Specific Optical Recording and Stimulation

Compared to electrical techniques that directly interface with the surrounding tissues, optical techniques are capable of sensing and modulating specific neural activity using viral targeting agents. In the case of photometric recording of neural dynamics, genetically encoded calcium indicators (GECI) are used to enable production of fluorescent proteins within the targeted cells that fluoresce in correlation to intracellular calcium concentrations dynamics. These fluorescent measurements often require large multimodal diameter fibers or bulky head mounted cameras that record fluorescent dynamics of specifically targeted neurons as shown in Fig. 4b. Recording capabilities using optical recording techniques can be expanded past the central nervous system to be utilized in applications to measuring oxygen saturation of targeted tissue which is critical in understanding areas of tissue perfusion and its effects on neural activation and wound healing. In the case of optogenetic stimulation, ion channels are modified to enable conformation changes with light sensitive proteins or opsins that allow for ionic current flow through the cell membrane that can stimulate or inhibit cell activation. Optogenetic techniques can also extend past use in the nervous system and have shown to be capable of restoring visual functions in animals with retinitis pigmentosa, control of seizures, and cardiac pacing. Optogenetic techniques are relatively new and have recently seen their use in clinical trials to treat blindness and treatments for various psychiatric and neurological disorders. However, current technologies are limited requiring the use of optical fibers that are large in diameter and stiff causing glial scarring and motion artifacts during recording thus limiting experimental paradigms and application in studying freely behaving animal subjects that can significantly improve our understanding of behavioral circuits.
1.3.3 Microfluidic Pharmaceutical Delivery

Device capable of delivering pharmaceuticals to target areas allow for unique opportunities that can be combined with optical modalities for light dependent activation of pharmacological agents or slow release of pharmaceuticals for pain management as shown in Fig. 4c. Conventional delivery systems often rely on syringes, however for applications that require steady release of pharmaceutical agents micropumps are used. These pumps are tethered and bulky which provide disadvantage to mobility and increase risk of infection. By miniaturizing these pumps using thermal actuation a delivery up to 90% of the fluid stored within the reservoirs can be released through mechanical compliant microfluidic tubes that minimize lessons compared to that of metal cannulas. However, the high-power consumptions that are required to thermally actuate the pump mechanism often require the use of large storage capacitors or battery powered systems. To reduce power consumption of fully implantable pharmaceutical delivery systems electrochemical pumps to generate hydrogen and oxygen to pump 1.5 µL of fluid using only 800 µW of power. Further improvement of drug delivery systems to be used in clinical studies will enable opportunities to combine sensing technologies to deliver treatment in a miniature point-of-care devices.

1.3.4 Chemical Sensing

Understanding the chemicals (dopamine, glutamate, serotonin, etc.) used to communicate within the nervous system is critical to understand the relevance in neural activities and brain functions. Among these neurotransmitters, dopamine is important in regulating behaviors such as motivation, reward, and reinforcement. Recent development using PEDOT:PSS coated diamond films as electrochemical sensors has enabled capabilities for direct recording of dopamine concentrations within the brain (0.1 µM - 10 µM) as shown in Fig. 4d. This sensor is transparent
allowing for capabilities in combination with optical techniques for simultaneous recording and modulation. However, these sensors require an elaborate fabrication process to deposit thin-film stacks and are prone to oxidizing thus reducing their sensitivity over time. These devices are highly specialized to enable direct measurements of dopamine concentrations used to dissect dopaminergic pathways within the limbic system and can provide a useful tool in understanding behavioral circuits and understanding neurological diseases such as Parkinson’s and neuropsychiatric disorders such as depression.

1.3.5 Thermometry and Thermal Flowmeters

Precision thermometry of targeted organs provides useful insight in vascular perfusion and circadian rhythm that can provide clinically relevant information in cardiovascular health, cognition, and wound healing as shown in Fig. 4e. Traditional methods for thermography include infrared cameras or wired thermometers which are important in certain applications but do not offer ability to continuously measure millikelvin levels of temperature changes of targeted organs deep within the tissue. Further development of thermography techniques has enabled the use of thin films that rely on the temperature coefficient of resistance of deposited (10 nm) gold with a trace width of 100 nm that allow for low thermal mass (< 10 mJ cm⁻² K⁻¹) of the sensor and their ability to form intimate contact with their target organ. These thin films are also capable of delivering heat to the target organ to measure thermal conductivity of tissue and measure the flow of bio-fluids. However, these films require clean room manufacturing which are expensive and susceptible to motion artifacts that can change resistance through mechanical strain of the sensor. Development of sensors using low-cost scaling manufacturing methods are required to meet demands to study large populations that can provide useful insight in vascular perfusion.
associated with trauma and wound healing to develop clinical tool to provide a point-of-care for at home monitoring.

1.3.6 Direct Strain Measurement

Mechanical strain through weigh bearing activities that generate tension, compression, and torsional forces plays an important role in the development of bone mass and structural architecture\textsuperscript{104,105}. Despite importance of technologies to directly measure bone strain, knowledge of the musculoskeletal system is limited. Standard approaches of measuring direct bone strain which include the use of wired resistive strain gauges as shown in Fig. 4f that have allowed for a greater understanding of bone health and diseased states such as osteoporosis and osteopenia where fragility fractures are more likely to develop with repetitive micro-strains\textsuperscript{10,11}. However, these systems are susceptible to changes in wire resistance that can affect data readouts as well as delamination from the bone from forces exerted on the wires and bio-degeneration of the cyanoacrylate or polymethylmethacrylate adhesive\textsuperscript{106–108}. To improve applications of implantable strain sensors, devices must allow for long term operations that allow the study bone development which can take up 6 to 10 months which is important for the development of point-of-care systems to monitor patient’s bone health during post-operation and drive effective therapeutic treatments to improve rehabilitation of the musculoskeletal system\textsuperscript{109}.
2. RESULTS: OPPORTUNITY FOR INTERMATE INTERFACE WITH TARGET ORGANS

2.1 Photometric Recording

Recording neural activation with cell specificality in freely behaving subjects yields significant insight between functions of the brain and their effect on animal behaviors. Current methods for measuring calcium dynamics rely on optical fibers to simultaneously excite genetically targeted neurons with GECI and record their fluorescent activity, described in section 1.3, which can significantly impact animal behaviors, mobility, and limit experimental paradigms to open two-dimensional areas. Wireless systems have eliminated the need for optical tethers, however battery powered devices that are large and bulky impact behavioral outcomes and are limited in their operational lifetimes. The photometry system, introduced in APPENDIX A\(^1\), avoids limitations of tethers and battery powered technologies, described in section 1.1, enabling the miniaturization of these devices to be fully implantable with injectable probes that allow for photometric recording in the deep brain as shown in Fig. 5 (top right). This new class of fully implantable wireless, battery-free photometry devices allow for the uninterrupted interrogation of specific neural dynamics of freely behaving subjects by effectively utilize MRC for WPT. Here I have decreased the size to 10.5 mm × 7 mm which is 170% smaller than previous wireless battery-free systems\(^41\). I have optimizing dimensions of the antenna enabling power harvesting capabilities (17.64 mW) in a 18 cm x 12 cm cage within an usable digital voltage range. The antenna design overall increases efficiency of the rectified power thus minimizing overall device heating\(^1\). This antenna design also shows optimal power harvesting per antenna area of 42.37 mW/cm\(^2\) which is a factor of 3.5 increase from previous battery-free devices using MRC\(^1,41\). This device exceeds
requirement set for WPT in photometric devices for small animal subject such as mice as described in section 1.1.1. I have also developed communication methods and optimized communication protocols that is capable of sufficiently streaming real-time data at 27 Hz with 12-bit resolution using IR communication. These data packets are captured by an array of five IR receivers distributed above the cage lid to improve communication stability within the volume of the cage to match that of Bluetooth systems\textsuperscript{1}, as described in section 1.1.2. I have also combined effective use of thin flexible device structures and soft biocompatible materials, described in section 1.2 to minimize damage to surrounding tissue. By leveraging the use of a wireless battery free system with soft flexible designs we show that there is no significant difference in animal behavior or mobility before and after implantation while allowing for full recovery of both skin and fur in only 20 days. The unique probe allows the recording of neural dynamics using a co-located micro light emitting diode and photo diode laminated with an organic thin film filter allows for miniaturization of the probe cross section compared to that of conventional optical fibers, thus minimizing damage to surrounding soft tissues. With the ability to be scalable for manufacturing using monolithic designs and consumer available components, suggests a widespread potential for adoption in the neuroscience community to study long-term neural activation with cell specificity and their effect on animal behaviors introduced in APPENDIX A\textsuperscript{1}.

### 2.2 Deep Brain Electrical Stimulation

Implantable DBS systems are currently being utilized for clinical treatment of neurological diseases such as Parkinson’s and Alzheimer’s. However, the knowledge of long-term operation of DBS for use in modulating learning behaviors and their use in therapeutic treatment of motor disorders are not well understood. Investigation of these neural circuits are accomplished in small
animal models to understand fundamental mechanisms of the brain. However current chronic stimulation devices require frequent handling to manage tethers that restricts experimental paradigms or charge batteries that removes the subjects from its natural habitat. These compromise insights to understand fundamental mechanisms of the brain, therefore I have developed a fully implantable, wireless battery free platform, introduced in APPENDIX B, allows for the chronic DBS in rodents with control over stimulation parameters in real time as shown in Fig. 5 (bottom right). I have optimized antenna designs though iterative imperial electromagnetic designs based on insight provided by work represented by chapter 2.1 by adjusting for trace space, width, and number of turns (6-turn dual-layer design, 70 µm trace width, 70 µm trace space). To improve power harvesting in larger rodents to expand testing paradigm this device is capable of wireless communication to control for stimulation timing and amplitude in freely behaving subjects without additional components or power. This allows devices to be highly miniaturized to study freely moving subjects in large cage designs up to 999 cm² without impacting their mobility or behavior, which has not yet been possible for current wireless battery free systems for electrical neuromodulation, as described in section 1.1.1. The device antenna is optimized to deliver high voltage compliance stimulation (5.5 V) within the volume of the cage and capable of harvesting 18 mW with additional capacitors to buffer power during stimulation events. Due to the higher voltages, thermal distribution layers are added to minimize localized heating (< 1 °C) and have been designed to meet safety standards (of the American Association of Medical Instrumentation). To minimize power consumption and heating, I have also implemented a communication protocol to convert standard microcontrollers with EEPROM to be capable of receiving wireless communication without additional hardware or power consumption. This system meets requirements for WPT and communication allowing for continuous operation with
digitally programmable control (8-bit) over stimulation parameters (period, pulse width, amplitude) with a range of physiological relevant stimulation capabilities (> 20 µs pulses, > 25 kHz frequency). By adjusting the stimulation parameters this systems is capable of modulating neural activity within the central and peripheral nervous system to control behavior\textsuperscript{2}, block pain signals\textsuperscript{110}, or be use in therapeutic treatment of spinal cord injuries\textsuperscript{111}.

This system provides opportunities to expand current paradigms to study the long-term use of deep brain stimulation and testing stimulation dosing which have not been possible with current technologies that rely on tethers or battery powered systems. I have also investigated the use of various encapsulation methods for wireless battery free systems, showing Parylene encapsulated devices allow for operation lifetimes in animal on the order of years, compared to other encapsulation methods such as polyurethane and platinum cured silicone with lifetimes of less than a month, as described in section 1.2.2. The micro-electrode interface allows for low impedance contact with the brain tissue using surface engineered, platinum electrodes using biphasic stimulation to reduce charge injection currents while stimulating specific brain regions, described in section 1.3.1. Here I controlled the tri-state (High, Low, High Impedance) of the µC digital outputs to reverse electrochemical processes at the electrode interface by reversing polarity during biphasic stimulation between pairs of electrodes. The device is also designed to allow for low-cost manufacturing utilizing off-the-shelf components and features the ability to connect with customize electrode designs to improve utility and rapid dissemination of these systems in the neuroscience community. This system has demonstrated its application to study long-term interconnected regions of the brain stimulating the vibrissal primary somatosensory cortex and its effect on the vibrissal primary motor cortex and to study chronic stimulation of the medial
forebrain bundle in freely moving subjects release dopamine while evoke characteristic head motion for over 36 days, discussed in APPENDIX B².

Figure 5. Integration of hard electronics with biocompatible, soft, and flexible materials in a single subdermally implantable device to allow intimate interfaces with targeted soft tissues⁷. Adapted/Translated by permission from Springer Nature Customer Service Centre GmbH: Springer Nature Biomedical Engineering, Wireless and battery-free technologies for neuroengineering, Sang Min Won, Le Cai, Philipp Gutruf & John A. Rogers, March 8, 2021.

2.3 Osseosurface Electronics

With advances in soft flexible material and sensor platforms, we now have the opportunity to interface with parts of the body that are largely understudied such as the musculoskeletal system. Bone mounted systems offer tremendous advantages for chronic bio-interfaces due to recent the development in permanently bonded strain gauges that allow for a stable surface for sensing and stimulation interfaces that can adhere for years, described in section 1.2. Here I introduce a new class of wireless, battery-free devices that are capable of interfacing with the musculoskeletal system in APPENDIX C³, with soft, thin device mechanics with a multimodal probe design, described in section 1.3, designed for optogenetic stimulation and high-fidelity thermal recording.
and strain recordings as shown in Fig. 5 (left). I have optimized antenna designs to balance WPT and wireless communication. Here the device is capable of harvesting 16.13 mW in a large experimental arena in the worst case, while providing voltage supply capable of powering digital electronics ~2.2 V which is sufficient to enable continuous operation within the volume of the treadmill cage, which solves the challenges described in section 1.1.1. This device is capable of communication up to 87 Hz with 14-bit resolution for both temperature and strain which is sufficient to record the strain generated by the impact forces during normal gait. This has been achieved by designing a custom communication protocol that transmits blocks of strain and temperature recordings that are later post processed and automatically aligned to minimize loss of data during communication collision. This has allowed for real-time streaming of high fidelity recording that allow for direct observation of strain as the animal walks on the treadmill, thus solving current limitations described in section 1.1.2. Here, I have demonstrated long term adhesion on the bone using surface engineered calcium phosphate ceramic particles that allow the probe surface to permanently bond onto the bone. Overall, this system, enable long-term studies of the musculoskeletal system which have not yet been possible due to lack of sensing modalities and proper bone adhesion in fully implantable formfactors that enable long term experimentation in small animal subjects. This system also highlights capabilities of osseousurface device for exploratory research and utility as a diagnostic and therapeutic platform. discussed in APPENDIX C3.

3. CONCLUSION AND FUTURE RESEARCH

Fully implantable, wireless, battery-free implantable medical devices introduced here have shown capabilities matching or exceeding current tethered approaches while expanding
experimental paradigms to explore understudied regions of the body such as the nervous system and musculoskeletal system. By integrating engineering concepts in advanced WPT schemes, wireless communication methods, mechanically engineered structures, soft materials, and modalities for sensing and stimulation, I have advanced current capabilities of fully implantable, wireless, battery-free systems to effectively study freely-behaving, small animal subjects with intermate organ interfaces that allow for high-fidelity readouts and precise stimulation across many parts of the anatomy. These capabilities foreshadow opportunities for further device development that can leverage these engineering concepts to develop new tools for exploratory research and clinically relevant therapeutic tools.

From these engineering concepts, I have advanced current capabilities of subdermally implantable, wireless, battery-free systems to improve understand of long-standing questions about the nervous and musculoskeletal system. First, I have developed, and optimized antenna designs specifically for subdermal implantation in adolescent mice that allow for cell specific recording in deep brain structures for chronic, uninterrupted studies in multiple freely behaving subjects which previously was limited by battery or tethered platforms. Using a wireless, battery-free platform provides significant insight to the long-term, uninterrupted development of deep brain structures and neural dynamics during social interactions between subjects. This device platform also expands current experimental paradigms that are limited by tethered or battery powered systems by providing ethologically relevant environments to deciphering functional neural pathways and their impact on animal behavior. These antenna, mechanics and system architectures also provide a platform for future miniature wireless battery-free devices implanted in small animal subjects that are optimized for both power harvesting efficiencies and size.
Secondly, I have optimized power harvesting capabilities of these miniature devices to expand experimental paradigms in larger cages to study larger rodents such as rats and their behaviors during electrical stimulation of deep brain structures with surface engineered micro-electrodes for precise targeted stimulation. I have also developed protocols to be used with all EEPROM enabled microcontrollers to allow wireless communication for precise control over stimulation parameters to study deep brain stimulation dosing for therapeutic treatment of movement disorders without additional hardware or power requirements. The communication platform designed here enables existing and future devices with microcontrollers and EEPROM to digitally receive commands for the programming of stimulation parameters.

Third, I have developed electronics to enable osseousurface integration of strain and thermal sensors and optogenetic stimulation capabilities to enable long-term, high-fidelity data streams to monitor and improve bone health. I integrated and optimized NFC communication scheme to enable the transmission of high-fidelity strain measurements on the bone during gait which is also compatible with smartphones for future translation of these devices toward large animal models and eventually towards at home patient monitoring. We are able to show direct relationship of the device growing directly on the bone over a period of a few days after implantation. Currently these devices have shown capabilities to improve mechanistic studies of osteogenesis and pathogenesis of musculoskeletal diseases.

This system also provides a platform for future work on developing fully implantable wireless system requiring greater communication distances for larger subjects such as rats and sheep in highly mobile areas. Leveraging combinations of the engineering concepts I have developed, fully implanted device that allow for further understanding of the functional mechanisms in understudied areas of the body such as the nervous and musculoskeletal system are
now possible in freely-behaving, small animal subjects. The optimization of wireless power transfer, communication, flexible mechanics, and soft materials specifically used for subdermal implantation in small animal subjects can be leveraged to enable implantable platforms with other sensing and stimulation functionalities. As the technical maturity of these devices evolves, opportunities to develop integrated networks of implantable system, illustrated in Fig. 5. will allow for communication between implanted nodes for real time insight of multiple organ systems which will significantly improve our understanding of functional mechanisms of the body and further development of new therapeutic and diagnostic tools that directly treat patients. This will require future work in both wearable and implantable systems that enable WPT and wireless communication with implanted nodes that target specific organs. Here these device nodes can be used in a close loop system that enable both sensing and stimulation. This approach will enable autonomous systems that automatically treat chronic illness, manage disabilities, and offer advanced insight into fundamental mechanisms of human physiology without the need for manual intervention.
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APPENDIX A: WIRELESS, BATTERY-FREE SUBDERMALLY IMPLANTABLE PHOTOMETRY SYSTEMS FOR CHRONIC RECORDING OF NEURAL DYNAMICS

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Contributions by Alex Burton: Designed, fabricated, populated, and tested the electronic circuit. Optimized antenna design for both small adolescent and adult mice. Optimized amplification circuit to record fluorescent within physiological ranges of calcium in mice. Improved wireless communication data rate by optimizing NEC communication protocols and tested effective spatial distribution of IR receivers. Designed alignment tools to guide electrode placement. Designed benchtop tests, in-vivo research experiments, collected in-vivo data, analyzed data, and wrote the paper.
Abstract

Recording cell specific neuronal activity while monitoring behaviors of freely moving subjects yield data that provide some of the most significant insights into brain function. Current means for monitoring calcium dynamics in genetically targeted populations of neurons rely on delivery of light and recording of fluorescent signals through optical fibers that can reduce subject mobility, induce motion artefacts, and limit experimental paradigms to isolated subjects in open, two-dimensional spaces. Wireless alternatives eliminate constraints associated with optical fibers, but their use of headstages with batteries adds bulk and weight that can affect behaviors, while offering only limited operational lifetimes. The systems introduced here avoid limitations of both types of technologies, by combining highly miniaturized electronics with injectable photometric modules in a class of fully wireless, battery-free photometer that is fully implantable subdermally to allow for the interrogation of neural dynamics in freely behaving subjects, without limitations set by fiber optic tethers and with operational lifetimes set by traditional power supplies. The unique capabilities of these systems, together with their compatibility with magnetic resonant imaging and computed tomography and the ability to manufacture them with techniques in widespread use for consumer electronics, suggest a potential for widespread adoption in neuroscience research.

Significance

Monitoring neuronal activity with cell specificity in freely behaving subjects is a critical tool gain insight into the underpinning mechanisms of the brain. Dynamics of genetically targeted neuronal populations can be recorded through calcium indicators, however current tools are only available with tethers or large and heavy externalized headstages, significantly impacting
behaviors of the test subject and limiting experimental paradigms to simple two-dimensional arenas. Here we introduce highly miniaturized, wireless, battery-free and subdermally implantable photometry systems that bypass current limitations and enable chronic recording of neural dynamics in ethologically relevant environments with no impact on test subject behavior. This tool has broad utility in neuroscience research and can enable fundamentally new experiments with insight in neuronal dynamics and simultaneous behavior studies with high fidelity.

1. Introduction

Deciphering functional neural connections across the central nervous system is critically important in efforts to establish an understanding of the underlying working principles, an overarching goal in modern neuroscience. Advanced tools that can record cell specific signals with high spatiotemporal fidelity in freely behaving subjects are essential to research in this field of study. Current means for recording such signals relies on genetically encoded calcium indicators (GECIs) capable of targeting cells with high specificity (1–3). Fluorescent signatures from these indicators follow calcium transients associated with cell activity. Practical execution of this experimental method currently relies on telecommunication grade optical fibers that deliver optical stimuli to genetically targeted cells and relay spectrally separated fluorescence emission to external detection electronics for filtering and digitalization. Such photometric systems can reveal, for example, neurological pathways associated with aggression, movements, and social interactions in behaving animals (4–6). The fiber optic tether represents, however, a major drawback that restricts mobility (7), thereby significantly constraining experimental paradigms to those that do not require three-dimensional (3D) arenas or studies of multiple subjects in pairs or social groups. The tether can also induce motion artefacts that limit signal fidelity and induce micromotions
detrimental to the neuronal interface via formation of glia scaring (8, 9). Recent work on wireless photometers avoid these drawbacks, but these systems rely on headstages with mounted batteries whose bulk and weight cause unwanted effects on behaviors in small animal models and set limits on operating lifetimes (10). An ideal alternative would involve an ultraminiaturized, lightweight, fully subdermal implant, with capabilities for interrogating neural dynamics with device lifetimes that exceed those of the test subjects themselves. Here, we present such a system, in the form of a photometric system that couples compact electronics with strategies for wireless power delivery and data transmission, capable of use in some of the smallest animal models, including young mice. Compared to previous work, the devices reported here eliminate the use of batteries and tethers, thereby allowing for continuous and chronic recording of neural activities, in a platform whose small size and weight enable fully subcutaneous implantation. This technology relies critically on strategies for wireless energy harvesting with efficiencies that exceed those of previous work by several times (11, 12). The use of off-the-shelf components facilitates scalable production, with a nonmagnetic construction that allows compatibility with magnetic resonance imaging (MRI) and micro-computed tomography (µCT) imaging, for advanced structural and functional analysis.

2. Results

2.1 Miniaturized wireless photometry design.

Fig. 1a displays a layered rendering of the miniaturized implant, designed for recording GECIs dynamics in freely moving subjects using an injectable photometry probe with co-located microscale inorganic light-emitting diode (µ-ILED) and photodetector (µ-IPD) for fluorescent stimulation and recording, respectively, both interfaced to a microcontroller (µC).
A modulated infrared (IR) LED provides the basis for wireless data communication to a separate photodetector via transmission through the skin and fur of the animal. The system receives power wirelessly by magnetic resonant coupling (13.56 MHz) between a primary antenna that encircles the experimental arena and a millimeter-scale receiving antenna on the implant. Constituent layers include a thin dual-sided flexible circuit board comprised of rolled annealed copper layers separated by a polyimide film, structured using a high-resolution laser ablation system (LPKF U4). The thin construction leads to a level of mechanical flexibility that allows
conformal contact with the curved surface skull and minimizes tissue damage during surgical implantation (12–14). Each electronic component features a small footprint, with high performance, low power operation. All components, listed in Fig. S1, are commercially available, to enable functional reproducibility and alignment with capabilities in scaled manufacturing. The design features an injectable component with a serpentine interconnect geometry to facilitate articulations of the probe during positioning to target a brain region of interest (10).

The operating principles, schematically shown in Fig. 1b, can be supported by electronic components that consume an area of only 0.21 cm², a reduction by 3.5-fold in comparison to previously published examples (11, 12), as shown in detail in Fig. S1. The circuit enables excitation of fluorescence by a µ-ILED and amplified detection of this fluorescence by a µ-IPD, signal digitalization, and wireless transmission. All of these operations rely on power wirelessly received through coupling to a primary antenna that encloses the experimental arena and connects to a radio frequency (RF) amplifier (NeuroLux inc.) operating at 13.56 MHz. (11) The harvested power is rectified and regulated by a low drop out voltage regulator (LDO, 3.3 V). The stabilized voltage powers a µC that controls the µ-ILED for excitation of fluorescence from the GECIs and a transimpedance amplifier circuit for amplification of photocurrent signals from the µ-IPD. Both optical components mount on the tip end of a thin, flexible injectable probe. Amplified signals are subsequently digitalized by a 10-bit analog-to-digital-converter (ADC) integrated in the µC and oversampled at 12-bit precision with 4 averages and then transmitted using an IR LED (950 nm) with a modulated carrier frequency of 57 kHz and ON/OFF keying with data rates of up to 27 Hz and a radiosity of 0.542 mW/mm². Passage of this light through the scalp attenuates the light by an amount that depends on the species and phenotype, and can range from 47% transmission in albino CD1 IGS mice (12) to 30% for Swiss Webster (albino) species with white fur to as low as
~1% for C57BL/6 mice with black fur (Fig. S2 (a)), resulting in a radiosity of 8.67 \( \mu \text{W/mm}^2 \). Attenuation of the IR light predominantly occurs in the fur. Removing the fur by shaving leads to transmission values of ~70-80% in all species and subjects examined (Fig. S2 (b)).

The IR receivers (TSDP34156, Vishay Semiconductors) are highly integrated devices in small packages (5.6 mm x 6 mm x 7 mm) that include a lens system, an optical filter (900 nm-1025 nm), an automatic gain control, a bandpass filter that reduces noise at frequencies other than the carrier frequency, and demodulator circuits that pass only the digital data signal to an external \( \mu \text{C} \). The lower detection threshold is 0.00015 \( \mu \text{W/mm}^2 \) even for background ambient irradiances of over 1 \( \text{W/m}^2 \). Transmission is most efficient in direct line-of-sight to the receiver, but in practice the system can operate without interruption across a wide range of environments that include opaque enclosures or 3D features based on indirect IR reflections.

Receivers positioned in the center of the experimental arena (30 cm x 20 cm) at a height of 12 cm indicated by red circles in Fig. 1c, each providing direct line-of-site to the implanted device for common types of behavior studies such as operant conditioning chamber (15), open field (16), light dark assay (17), elevated plus maze (18), place preference cage (19), water maze (20), and rodent treadmill (21). The signal is decoded using a NEC protocol in real time through an external system (ATmega328). The transmitted byte string includes fluorescent values that are time-stamped and sent via a serial universal serial bus (USB) connection for storage and analysis.

Stable operation of the \( \mu \text{C} \) relies on effective wireless power transfer, which depends strongly on the size and geometry of the receiving antenna, within the constraints set by the available subdermal space on the head of the animal. The inset of Fig. 1c shows the architecture of two photometry device variants placed on 3D printed models of mouse skulls. The large photometry device (13.5 mm x 10 mm, 59 mg), applicable to large mouse strains, can support
operation across large experimental arenas (780 cm², at 9W RF power). The small design (10.5 mm x 7 mm, 45 mg) can apply to small strains and young mice in comparatively small (650 cm², at 6W RF power) arenas, although extendable to larger sizes with the additional of transmission antennas. This small platform can, in fact, enable experiments in animals such as adolescent mice that are not possible with conventional optical fiber approaches (10, 14).

2.2 Photometry probe design.

Fig. 1d displays an exploded view schematic illustration of the probe. A flexible polyimide film (thickness 75 µm) serves as a substrate for electrodes and interconnections formed with a patterned bilayer of Cu/Au (thickness 250 nm/250 nm). The tip of the probe includes a µ-ILED (270 µm x 220 µm x 50 µm) and a µ-IPD (300 µm x 300 µm x 100 µm) placed adjacent to each other. A narrow-band optical filter (400 µm x 400 µm x 80 µm) formed with an organic dye (ABS 473, Exciton) photolithographically defined and deposited over the µ-IPD rejects the light from the µ-ILED (468 nm) and passes light at the wavelength (523 nm) of fluorescence from the Ca²⁺ dye (Oregon Green 488 BAPTA-2). Fig. S3 presents a series of steps for fabricating the probe.

This probe connects to the main electronics body via a 250 µm wide serpentine structure designed to provide an effective level of stretchability that maintains strains in the metal layer that lie below the yield strain, thereby allowing for sustained elastic behavior without disrupting the electrical connectivity (22, 23) (Fig. 1e). With this arrangement, surgical placement of the probe to reach any position within the opening of the implant is possible, to target various brain regions. The probe inserts vertically into the brain, as shown in Fig. 1f. The thickest point of the electronics module, after encapsulation, is 1200 µm at the position of the µC, comparable to that of other types of wireless, battery-free subdermally implantable devices (12, 14, 24).
Fig. 2a presents a colorized scanning electron microscope (SEM) image of the tip end of the photometry probe and a photograph of the integrated system. The peak emission wavelength of the μ-ILED is 468 nm (Fig. 2b), which matches the fluorescence excitation requirement of many important GECIs, including GCaMP6 (2, 3). Characterization of the μ-IPD under 1-sun illumination (broad band light illumination) serves as a benchmark. The μ-IPD shows a short-circuit current ($I_{sc}$) of 2.26 μA, an open-circuit voltage ($V_{oc}$) of 1.59 V, and a fill factor of 78.5% (Fig. S4a). The relationship between $I_{sc}$ and the irradiance of the input light determines important characteristics of the μ-IPD, such as the responsivity. The $I_{sc}$ of the μ-IPD shows linearity with both simulated solar illumination and green light from an LED light source, with a responsivity of 39.32 μA/W under white light (Fig. S4b and S4c). The organic optical filter passes the green fluorescence signal and rejects the excitation blue light. Specifically, this filter has less than 1% transmittance for photons with wavelengths from 465 nm to 497 nm (Fig. 2c). As a result, the external quantum efficiency (EQE) of the μ-IPD coated with this filter decreases to 0.004% at 468 nm (Fig. 2d) and moderately decreases from 16% to ~5% at 520 nm. The result successfully minimizes the influence of the excitation light on the fluorescence signals.
Figure A-2. (a) Colorized SEM image of the photometry probe (Orange, polyimide; blue, µ-ILED; green, µ-IPD and optical filter). (b) Normalized emission spectrum of the blue µ-ILED used for optical stimulation. (c) Transmission spectrum of an optical filter composed of layer of a photodefined epoxy (80 µm, SU-8) doped with 1.5 wt% of an organic dye as a narrow band absorbing filter. (d) EQE spectra of a µ-IPD with (red) and without (black) an optical filter. The blue and green areas highlight EQEs under the maximum µ-ILED emission (blue) and GCaMP6f fluorescence (green) wavelength regions, respectively. (e) Angular dependence of the rejection ratio of blue (462 nm) to green (520 nm) illumination. (f) Response of the wireless photometry device to an external green light source (520 nm). (g) Fluorescence recording with standard errors for solutions with different calcium concentrations (0.0625 µM - 32 µM) and calcium dye (12.5 µM, Oregon Green 488 BAPTA-2). (h) Simulated spatial distribution of fluorescence fluence at a Ca²⁺ concentration of 32 µM and an input optical power of 0.5 mW. (i) Acceptance profile with 1% and 10% intensity contours captured by the µ-IPD at 520 nm. (j) Fluorescence density profile captured by the µ-IPD, obtained by the product of (h) and (i). (k) Comparison between simulated and recorded photocurrents corrected for stray light transmission through the organic filter. The simulations assume perfect blue light rejection.
The filter provides Lambertian transmission properties between 10°-170° from the surface of the µ-IPD. Fig. 2e illustrates the rejection characteristics, as defined by the ratio of the photoresponse to blue (462 nm) with respect to the green (520 nm) LEDs at equal irradiance (0.162 µW/mm2). The average rejection ratio at all tested angles is 3±0.3%, suitable for high fluorescence yields in GECIs such as GCAMP6. Details are in the Methods section. For common in vivo instances, the fluorescence yield is ~4% and ~60% for Ca2+ -free and -saturated environments, respectively, resulting in high-contrast changes in signal from the µ-IPD (3, 25). The wireless photometry device has a responsivity of 0.665 µA/W to green light (520 nm), with a highly linear (R² = 0.9997) response to irradiance at this wavelength, as shown in Fig. 2f. A fluorescent dye (Oregon Green 488 BAPTA-2; 12.5 µM) serves as a benchtop analog of GECIs for evaluating the fluorescence in an aqueous solution with a calcium concentration similar to that of natural intracellular calcium dynamics (typically 0.05 - 0.1 µM when at rest and up to 10 µM (26) when stimulated). Photometric recordings of the fluorescence with this testing approach indicate the ability to detect Ca2+ concentrations between 0.0625 to 32 µM, as shown in Fig. 2g with an average standard deviation (14.24 a.u.). Tests with the µ-ILED disabled show a baseline dark current of < 22 pA, which is three orders of magnitude smaller than the photocurrent at the lowest calcium concentration.

Monte-Carlo simulations yield insights into the optical performance of the system. Calculations with a scattering coefficient of 10 cm⁻¹ in a scenario dominated by forward scattering (dissymmetry factor of 0.85) define profiles of the µ-ILED emission at 470 nm with an optical input power of 0.5 mW, as in Fig. S5a. For a 12.5 µM concentration of dye (Oregon Green 488 BAPTA-2), where the absorption coefficient is µa-OGB-2 = 0.38 cm⁻¹ at 470 nm, this emission profile results in an absorption density and a total absorbed energy with distributions shown in Fig.
S5b and S4c, respectively. These results characterize the amount of excitation energy involved in the generation of fluorescence. Based on the well-established relationship between fluorescence irradiance and Ca2+ concentration (see Methods), these stimulation and absorption profiles yield corresponding fluorescent emission profiles, as shown in Fig. 2h and Fig. S5d for free Ca2+ concentrations of 32 μM and 0.06 μM, respectively. The acceptance profile of the μ-IPD with the organic filter can be simulated using Monte-Carlo with a test point source that captures the role of a fluorophore with isotropic emission profile and sweeps across the entire volume above the μ-IPD. With this profile, no more that 50% of the emitted power reaches the μ-IPD located at the lower hemisphere. The maximum response lies at the top surface of the μ-IPD, as shown in Fig. 2i and without the filter in Fig. S6a. The overall systemic response is determined by the overlap of the fluorescence profile and the μ-IPD acceptance, as observed in Fig. 2j. Therefore, the optimum fluorescence detection volume of the photometry probe corresponds to the region between μ-ILED and μ-IPD. In the fluorescence conversion and detection process, the illumination volume produced by the μ-ILED remains constant but the fluctuations in the concentration of Ca2+ result in different fluorescence powers emitted by the molecular dyes (or GECIs in biological system), for example see Fig. 2h and Fig. S5d. The photocurrent response measured by the μ-IPD corresponds to the fraction of the emitted fluorescence power that reaches the photodetector's area. Fig. 2k shows the simulated photocurrents in aqueous solution compared to experimental photocurrents at different Ca2+ concentrations (Fig. S6b shows same result but assuming perfect rejection of the blue light). The correlation with experimental results is excellent (R² = 0.9378; Fig. 2k). The current offset (~45 nA in the absence of Ca2+) observed in the experimental measurements is the result of several factors: 1) the blue light rejection ratio associated with the filter is not zero (~3%); 2) the illumination bandwidth (~50 nm) provides finite spectral overlap.
with the edge of the spectral response of the filter; and 3) blue light that travels directly from the side walls of the \( \mu \)-ILED to the \( \mu \)-IPD which contributes to the offset photocurrent. From Monte-Carlo simulations of the illumination profile, this photocurrent baseline can be estimated. The combined effects 1) and 2) produce 11.29 nA of baseline current; the effect of 3) produces 37.65 nA. As a result, the total baseline is \( \sim \)49 nA, comparable to that observed in experiment (45 nA).

2.3 Wireless power and mechanical properties.

Optimizing the power harvesting component of the system is an important goal in engineering design. The following presents configurations for two different harvesting antennas, with dimensions and layouts configured to match the sizes of different animals and arenas. Fig. 3a shows a small device that allows implantation on the space provided by the skull of an adolescent mouse (10.5 mm x 7 mm). The size is 170% smaller than previously published examples of wireless battery-free optoelectronic systems designed for other purposes (1 cm\(^2\)) (12), enabled by a dual layer antenna design optimized to deliver output power in a usable voltage regime thereby increasing efficiency of the rectification and voltage regulation system. Fig. 3b shows harvesting characteristics with increasing load in the center of a mouse 18 cm x 12 cm arena with a dual-loop primary antenna supplied with 3 W of RF power. Here, the minimum harvested power in this setup (17.64 mW, 8.75 V) occurs in the center of the arena, as in Fig. S7a. The receiving antenna features a dual-sided copper coil with 7 turns, 100 \( \mu \)m wide traces with spacings of 50 \( \mu \)m, for a Q-factor of 23.05 (Fig. S7b). Results of additional tests across a 28 cm x 28 cm arena with 10 W through the primary antenna appear in Fig. 3c, at two physiologically relevant heights, i.e. 3 cm which corresponds to the height of the head of the mouse during walking/running and 6 cm which
represents the height in the rearing position. The 18 cm x 12 cm and a 25.5 cm x 25.5 cm arena tested at 5 W show similar spatial variations at heights of 3 cm and 6 cm, as in Fig. S7c and S7d.

Figure A-3. (a-c) Highly miniaturized photometry device for implantation in small and young mice: (a) Photograph of the device balancing on a finger. (b) Corresponding power vs. load curve in the center of the 18 cm x 12 cm arena with RF input power of 3 W. (c) Spatially resolved energy harvesting capability of the miniaturized device in a 2 turn primary antenna around an arena with dimensions of 28 cm x 28 cm and 5 W RF input. (d) Photograph of the device balancing on a finger. (e) Corresponding power vs. load curve in the center of the 18 cm x 12 cm arena with RF input power of 3 W. (f) Spatially resolved energy harvesting capability of the large device in a 2 turn primary antenna around an arena with dimensions of 28 cm x 28 cm and 5 W RF input. (g) Time resolved current consumption of the device during sleep (yellow),
stimulation/recording (blue), and IR communication (red). (h) Harvesting performance of small and large devices in a 25.5 cm x 25.5 cm arena as a function of RF power input. (i) Harvesting performance of both devices in a 28 cm x 28 cm arena as a function of RF power input. (j) Angle-dependent ADC reading in the 18 cm x 12 cm arena at an input power of 6 W. (k) Height-dependent ADC reading in the 18 cm x 12 cm arena at an RF input power of 6 W. (l) Stress-strain curve of the serpentine interconnects.

Identical tests use a photometry device with comparatively large dimensions, shown in Fig. 3d, to exploit the area available on the skull of a fully-grown adult mouse. The layout of the copper harvesting antenna in this case consists of 6 turns, 100 µm trace width with 50 µm spacing, for a Q-factor of 20.46 (Fig. S7e). Fig. 3e shows the harvesting characteristics tested in an 18 cm x 12 cm arena, using setups similar to those described above, at an RF power of 3 W under increasing resistive loads. This large antenna yields increased harvested power, as shown in the center of the 18 cm x 12 cm arena in Fig. S7a (21.68 mW, 9.43 V). This system allows improved operation in large arenas, as shown in Fig. 3f, where the power harvesting capabilities follow similar spatial distributions as those of the small device, but with increased overall output. Further evaluations in the 18 cm x 12 cm and a 25.5 cm x 25.5 cm arena at an RF power of 5 W show similar spatial variations at heights of 3 cm and 6 cm in Fig. S7f and S7g.

Previously reported systems (25 cm x 15 cm arena, 4 W RF power) harvest 12 mW (12), corresponding to a harvesting capability (normalized by device size) of 12 mW/cm². The small device (0.57 cm²) harvests 24.15 mW with the same arena and conditions (25 cm x 15 cm, 4 W RF power) resulting in a harvesting capability of 42.37 mW/cm², an improvement of a factor of 3.5 that follows mainly from an improved Q factor, as shown in Fig. S7b and S7e.

Power consumption characteristics appear in Fig. 3g at a system voltage of 3.3 V. The behavior follows three distinct phases, i.e. system sleep, fluorescence sampling and data communication, marked in yellow, blue and red respectively. The peak power consumption is 10.37 mW during fluorescence recording. The sleep mode consumes as little as 119 µW, thereby
providing an effective means to reduce power consumption by reducing the sampling rate (Fig. S7h).

Electrical stability in the test arena and mechanical robustness of the electrical circuitry are critical in successful operation during chronic implantation in small animals. Reducing the power consumption and increasing the harvesting capabilities enhance the stability of operation during vertical misalignments that can occur during behaviors such as rearing and sleeping. A relevant metric, which we refer to as the safety factor, is the ratio of harvested power to the average power consumption of the device. This parameter allows for estimates of the maximum misalignment angles (27) that can be tolerated anywhere in the arena without operational interruption, using Fig. S7i. Fig. 3h shows the harvesting capability of both devices in the center of a 25.5 cm x 25.5 cm experimental arena typically used for a range of behavioral experiments (1, 3, 5, 14, 28). A dashed black line indicates peak power consumption of the electronics for both device sizes, with a 120% safety factor to account for reductions in harvested power due to angular misalignment (~25° with respect to the primary antenna). Here seamless operation of the implant can be maintained with RF powers of 5 W and 7 W respectively. These power levels are similar to those of other wirelessly powered (13.56 MHz) devices and are well within regulations approved for operation in public spaces (29, 30). Fig. 3i shows the harvesting capabilities of both devices in a large experimental arena (28 cm x 28 cm). Here operation cannot be sustained by the small device in the center of the arena, thereby limiting the operation for younger and smaller mouse models to arenas smaller than 25.5 cm x 25.5 cm. Possible solutions to extend the arena size involve tracking of the subjects with activation of the corresponding antennas powered by additional power amplifier devices (31).

Fig. 3j demonstrates operating conditions with a high safety factor (437%) for a large device in a dark room at various angles across an 18 cm x 12 cm arena with an RF power of 7 W,
for stable operation with misalignments as large as 70°. At insufficient power, communication loss occurs rapidly with minimal erroneous or corrupted data. As outlined in Fig. 3c and f, harvested power can fluctuate significantly depending on position in the experimental arena. An LDO actively regulates the output voltage to 3.3 V for input voltages between 3.45 V to 5.6 V. Utilizing regulators with low dropout voltages (~150 mV) and with over voltage protection using 5.6 V limiting Zener diodes, the device maintains stable ADC recordings with controlled illumination at all locations in the 18 cm x 12 cm arena shown in Fig. 3k.

Mechanical robustness, biocompatibility and flexible form factor are additional important considerations for successful deployment as a chronic subdermal implant. Mechanical isolation of the electrical circuitry from bending deformations exploits a potting process with a UV curable adhesive with a low level of shrinkage from the curing process (32). Further encapsulation with polyurethane (PU) (33) and a coating of polydimethylsiloxane (PDMS; ~10 µm thick) provides a biocompatible barrier to biofluids (34) and a degree of modulus matching with surrounding tissues (10) that reduces damage to adjacent tissues.

Fig. 3l shows the electromechanical characteristics of the injectable probe when stretched relative to the electronics platform. Here a high tolerance to strain allows for elongations of up to 1.5 cm in comparison to the relaxed state, corresponding to a tensile strain of 93.75% without disruption of electrical function. This elongation allows the probe to be bent out of plane and to be placed within the opening of the device to reach the desired brain region.

2.4 Device implantation and imaging.

The thin geometry, small dimension and lightweight nature of the device minimize loads on the animal and allow for seamless recovery shortly after implantation. Fig. 4a shows an animal
20 days post-surgery with skin and fur completely recovered. Elimination of the supercapacitor featured in previously published examples (12) decreases the size and bulk of the device, and removes all magnetic components. The result is a device that is compatible with both MRI and μCT imaging. Standard imaging procedures yield very little distortions, comparable to those of recently reported optogenetic stimulators that have significantly reduced electronic complexity (11).

Figure A-4. (a) Photograph of a mouse 20 days after implantation of a small photometry device. (b) Transverse CT reconstruction of the implanted device. (c) Sagittal CT reconstruction of a slice that illustrates the position of the implant. (d) 3D rendering that combines MRI (blue) and CT (grey and yellow marked device structures) results. (e) Transverse MRI reconstruction of the device illustrating imaging capability around the target area. (f) Sagittal MRI reconstruction of the device illustrating minimal distortion of the brain. (g) Post-processed sagittal CT scan with false color device structures (yellow). (h) Transverse MRI and CT overlay of the device indicating successful registration and low dimensional image distortion in both MRI and CT. (i) Co-registered Sagittal MRI and CT overlay.
Figs. 4b and c show µCT images of the electronics and photometry probe and their position with respect to bone structure. This imaging capability is especially valuable for rapid post-surgery analysis to validate implant targeting. Tissue contrast of the MRI images (Figs. 4d-f) shows no distortion in the target area. The results can be used to identify brain regions on a per animal basis, to eliminate effects of natural animal-to-animal variances and variability in surgical procedures. High dimensional accuracy can be seen in co-registered MRI and µCT images (Figs. 4d and 4g-i).

2.5 Post implantation recording and behavioral impact.

Experiments that exploit the electronic and mechanical features of these devices in live animal models demonstrate successful and reliable operation across a home cage (30 cm x 20 cm) with 8 W RF input (safety factor 137%), as evident by low loss of packets (Fig. 5a) each containing 12-bit integers. Here, evaluations of sampling capability use a test program that transmits incremental values to validate the IR communication link, as shown in the inset of Fig. 5a. A set of experiments that tracks the position of the animal throughout the arena and records packet loss as a function of position reveals that only 8 data packets drop during a typical 4.5 min test data segment. This level of performance is comparable to other low power wireless communication methods, including Bluetooth (35). Additional tests probe various locations of an implanted mice in an arena (30 cm x 20 cm) with recording times of ~23 mins. In a benchtop endurance test, the device located in the center of a home cage at 5 W of RF power, experimental results in Fig. S8 demonstrate continuous recording of an external green light source (0.38 mW/mm², 520 nm) pulsing at 1 s intervals for 24 h in a dark room with 0 packet loss and no change in signal magnitude or timing. This high level of robustness in data communication follows from a sensitivity in the receiver (0.15 mW/m²) and a wide field of view of 90° (Fig. S9a-b) for recording both line of sight
and indirect signals. Smaller arenas can be recorded with a single receiver while larger arenas benefit from multi receiver setups, as depicted in Fig.S8b where 5 receivers connected in parallel improve the detectivity.

**Figure A.5.** (a) Data transfer rates in a freely behaving subject (left) and test pattern designed to evaluate performance (right) (dropped datapoints marked in red). (b) Weight of animal as a function time after implantation. (c) Elevated plus maze experiment with overlay of traces of motion of both implanted and control mice. (d) Average velocity, time in the closed arm, time in the center and open arms, and number of transitions between closed and open arms of the mice in the elevated plus maze. (e) Open field experiment with overlay of traces of motion of both implanted and control mice. (f) Distance traveled, average velocity, and time...
spent in the center of the arena of the mice in an open field assay. (g) Light-dark room experiment with overlay of traces of motion of both implanted and non-implanted mice. (h) Time spent in the light room and number of transitions between the light and dark room of both implanted and control mice in a light-dark assay. (i) Weight of the pellet consumed during a 1 h feeding session. (j) Digital data collection of a mouse during a forced swim test in a 5-gallon cylindrical tank with data packets dropped shown in red.

Both large and small devices show no loss in performance for at least 2 months after implantation. Mice after surgery show recovery speeds comparable to other wireless and optically tethered photometry systems (10, 36, 37), as shown in Fig. 5b, with unrecovered mice in Fig. S10. Animal welfare is also not affected by either the surgery or the presence of the device, as indicated by the steady weight progression of the animal’s post-surgery.

As mentioned previously, the small photometry device (10.5 mm x 7 mm; 45 mg) can record neural dynamics in young mice (10 - 11 weeks). Tests of behavioral impacts on anxiety, locomotion, and feeding involve a cohort of implanted animals (n=5) and a control group (n=5). Anxiety and locomotion tests involve analysis of both movement and spatial preferences within open field and elevated plus maze assays (38, 39). Implanted mice automatically tracked in the elevated plus maze indicate no visible difference in anxiety or locomotion. Representative traces of an implanted and control subject are in Fig. 5c. Analysis of the cohort with unpaired t-tests between average velocity (p = 0.060), time spent in the closed arm (p = 0.595), time spent in both closed and open arm (p = 0.595), and number of closed and open arm transitions (p = 0.605) reveals no significant difference between the two groups, as summarized in Fig. 5d. The open field experimental paradigm with implanted and control animals also reveals similar behavioral patterns (Fig. 5e). Analysis of the cohort shows no increased levels of anxiety or impairment in locomotion. Furthermore, results displayed in Fig. 5f with implanted mice show no significant differences in total distance traveled (p = 0.298), average velocity (p = 0.299) or total time spent in the center of the arena (p = 0.477). Light-dark box experiments for analyzing anxiety compare the drive between exploration and aversion to open and brightly lit areas (40). Movement traces of
representative animals in Fig. 5g show no significant differences in behavior between subjects with implant and control. Analysis using unpaired t-tests of time spent in light arena (p = 0.540) and number of transitions between the light and dark areas (p = 0.563) are in Fig. 5h. The result reveals minimal anxiety levels in the implanted mice and the control group. Elevated plus maze, open field, and light-dark box assays indicate that the implant had no significant effect on the anxiety level or locomotive behaviors. Both control and implanted mice with prior access to food placed in a test arena (29.2 cm x 19 cm x 12.7 cm) exhibit feeding behaviors (41) with that show no significant differences through an unpaired t-test (p = 0.626) in 1 h total food intake between groups, as in Fig. 5i.

The untethered mode of operation coupled with small weight and quick recovery of the test subjects creates possibilities for recording in behavioral paradigms that are not possible with current technologies. Recording of freely moving mice during a forced swim test, shown in Fig. 5j, highlights compliance with 3D environments and reveals seamless recording of neural dynamics in arenas (707 cm²) that are difficult or impossible to address with tethered techniques (42) (10). A communication experiment using 5 IR receivers, analogous to previously described tests in the home cage (Fig. 5a), reveals that communication in aquatic environments have similar link quality, with only 1.5% of the data packets dropped during a recording period of ~10 min for the full duration of a forced swim test.

3. Conclusions

In summary, we demonstrate a fully subdermally implantable photometry system that enables indefinite interrogation of calcium transients (0.0625 µM - 32 µM) in a battery-free, miniaturized form factor for recordings in freely behaving subjects with low power consumption
(10.37 mW). The thin, low profile geometry facilitates subdermal implantation even in small rodent animal models such as young mice. Significant improvements in energy harvesting and in device geometry create opportunities in recording GECIs in freely behaving subjects with high flexibility in probe placement for broad ranging applications in the dissection of neural circuits. The platform is fully compatible with live imaging by MRI and μCT, and with manufacturing techniques adopted from the semiconductor industry. Behavioral experiments reveal that the implantation and operation of the device does not impact behavior of the subjects in a variety of scenarios including aquatic settings. Collectively, these features suggest widespread utility in neuroscience research.

4. Methods

4.1 Flexible circuit fabrication.

Pyralux (AP8535R, constituent layers 17.5 μm copper, 75 μm polyimide and 17.5 μm copper) served as a substrate. The copper traces, vias, and device outline were defined using a UV (355 nm) laser ablation system (LPKF, Protolaser U4) with subsequent ultrasonic cleaning (Vevor, Commercial Ultrasonic Cleaner 2L) for 10 min in flux (Superior Flux & Mfg. Co., Superior #71) and 1 min in isopropyl alcohol (MG Chemicals, IPA) and rinsing with DI water to remove oxidation and organic residue. Via connections were established manually with copper wire (100 μm) and low-temperature solder (Chip Quik Inc., TS391LT).

4.2 Photometry probes.

Fabrication of the photometry probe began with spin coating a thin layer of PDMS adhesive on a glass slide. A polyimide film (75 μm thick) served as the substrate for the probe, laminated
on the PDMS adhesive. Photolithography and a lift-off process in acetone defined patterns of Cu/Au (formed by electron beam evaporation, thickness \( \sim 500 \) nm) electrodes and interconnects on polyimide film. Spin coating and patterning a layer of photo-definable epoxy (SU-8 2002; Microchem) formed an insulating layer on the electrodes with selective openings for the \( \mu \)-IPD and \( \mu \)-ILED. In/Ag solder pastes (Indalloy 290; Indium Corporation) were placed on electrodes in the openings. Transfer printing delivered the electrode pads of the \( \mu \)-IPD (TCE12-589, Three Five Materials Inc.) and \( \mu \)-ILED (C460TR2227-0216, Cree Inc.) onto the pastes. A subsequent heating process at 150 \( ^\circ \)C for 1 min formed a robust mechanical and electrical connection between the \( \mu \)-IPD, \( \mu \)-ILED and Cu/Au electrodes. A spin coated layer of a narrow band organic absorber (ABS 473, Exciton) mixed into a photo-definable epoxy (SU-8 2100; Microchem) (1.5 wt\%) formed the optical filter on the \( \mu \)-IPD. Laser cutting defined the pattern of the injectable probe and dip coating in PDMS formed the encapsulation layer of the final device.

The pads of the photometry probes were cleaned using Kimwipes Low-Lint Wipers (34155, Kimberly-Clark Professional) with IPA. These probes were electrically joined to the flexible circuit via reflow low-temperature soldering. The connection between the probe and device was then cleaned with IPA to ensure adequate mechanical adhesion using UV curable glue (Damn Good, 20910DGFL) and cured under a UV lamp (24 W,10 mins) and subsequently baked in an oven (50 \( ^\circ \)C, 20 mins).

4.3 Electrical tuning, components, and coding.

Commercially available components were placed by hand and reflowed with low-temperature solder. A half bridge rectifier was built using 0201 components (0.6 mm x 0.3 mm) for the tuning capacitors, smoothing capacitor and low capacitance, low forward voltage Schottky
diodes (Skyworks Inc.). Tuning capacitors were chosen to produce the lowest voltage standing wave ratio at 13.56 MHz during reflection testing with a reflection bridge (Siglent, SSA 3032X, RB3X20). A Zener diode (Comchip Technology Corporation, 5.6 V) provided over voltage protection and a 3.3 V LDO stabilized the input voltage to the microcontroller. A low-power microcontroller (Atmel, ATtiny 84) controlled the timing of the activation of the µ-ILED, the readout of the µ-IPD, and infrared communication. The microcontroller software was programmed using a Tiny AVR Programmer with a USB interface before mounting on the flexible circuit. The blue µ-ILED current was set using a 1 kΩ resistor to control irradiance of the blue LED stimulations. An amplifier (Analog Devices, ADA4505-1) was used in a transimpedance configuration to convert the current output of the µ-IPD into a voltage which was then digitized. The digital signal was modulated (57 kHz) and transmitted using a current limited IR LED with a 0402 package (1.0 mm x 0.5 mm).

4.4 Encapsulation.

Devices were rinsed with IPA to remove any particulates. Components were fixed to the flexible circuit using UV-curable glue (Damn Good, 20910DGFL) subsequently cured under a UV lamp (24 W, 10 mins) and crosslinked in an oven (80 °C, 20 mins) to ensure adhesion of additional layers. Devices were then encapsulated via spray coating of PU (MG Chemicals, Premium Polyurethane Conformal Coating) and cured in an oven (80 °C, 1 h), followed by a second coating and oven cure (80 °C, 24 h). The devices were finally encapsulated with PDMS though dip coating and curing at 80 °C for 24 h.
4.5 Device electrical characteristic testing.

Voltage and power harvesting characteristics of the device in the center of an 18 cm x 12 cm arena (18 cm x 12 cm) powered with an RF power of 3 W were determined using a shunt resistor with voltage measurement (Aneng, AN8008). A shunt resistor (1.5 kΩ) was chosen to impedance match with the rectifier. Peak power measurements of location-dependent harvesting capabilities with dual loop antennas with dimensions of 18 cm x 12 cm, 25.5 cm x 25.5 cm, and 28 cm x 28 cm result in voltage readings that were used to calculate power output of the implants. Harvesting capabilities were tested at heights of 3 cm and 6 cm. The primary antenna was powered with RF input of 3 W and 10 W, and testing was performed in the center of the 3 arena designs. Measurements of stable operation were conducted at two heights in an 18 cm x 12 cm arena in a dark condition with an RF power of 4 W while collecting IR data using a single receiver located 20 cm above the arena surface. Current consumption of the device was recorded using a lab power supply (5 V) and a current meter (LowPowerLab, CurrentRanger) with an internal shunt of 10 Ω and cascaded amplifiers (Maxim Integrated, MAX4239) with acquisition of real time current consumption via an oscilloscope (Siglent, SDS 1202X-E). The angular dependence of the power harvesting capability was tested by varying the angle of the implants with respect to the arena floor from 0 to 90° using a rotational jig while monitoring ADC recordings in a dark room using the digital communication IR receiver (Vishay Semiconductors, TSDP34156).

4.6 Device strain-stress testing.

The device was mounted to a scale (Mettler Toledo, AB104-S) with both ends of the serpentine connected to a multimeter to measure electrical continuity. The probe connection side of the serpentine was then fixed to a fixture to adjust strain of the device. The serpentine structure
was adjusted to its base length and stress was recorded after stretching the device at 2.5 mm increments.

4.7 Functional ex-vivo testing.

Angle dependent rejection ratios of blue and green light through the thin film optical filter were tested by using green (520 nm) and blue (462 nm) LEDs directed at multiple angles (10°-170°) on the μ-IPD at the same irradiance (0.162 μW/mm²). The rejection ratio was then calculated as the average ratio of the irradiance measured at the μ-IPD. The photo-responsive characteristics of the μ-IPD were tested using a green LED (520 nm) with intensity controlled by a constant voltage power supply (RIDEN®, DPS3005) and calibrated by a spectral power meter (Thorlabs, PM100). In both tests, the photocurrent of the μ-IPD was pre-processed in the μC (amplification and digitalization) and transmitted wirelessly.

A stock solution of 64 μM calcium chloride (Sigma-Aldrich, MO, USA) in de-ionized (DI) water was prepared and used to produce two-fold serial dilutions ranging from 0.0625 μM to 32 μM with a final volume of 0.24 mL. A 3 mM stock solution of Oregon Green (488 BAPTA-2, Thermo Fisher Scientific, NH, USA) in anhydrous dimethyl sulfoxide was mixed with each calcium solution to produce 12.5 μM concentration of dye across each vial. The solutions of dye and calcium were lightly vortexed in a vial to ensure adequate mixing prior to data acquisition. The photometry device was powered using a dual loop antenna encircling an 18 cm x 12 cm arena and powered with RF at 3 W in a dark room to prevent optical noise. The photometry probe was inserted into each test solution and readouts were recorded. After each test, the probe was removed from the solution and lightly rinsed of excess solution using DI water before recording the next set of data.
4.8 Fluorescence and photocurrent numerical calculations.

Detailed information on fluorescence and photocurrent numerical calculation methods is available in supplemental Text ST.1.

4.9 Surgical procedure.

All procedures were approved under Northwestern University and University of Arizona Institutional Animal Care and Use Committee. 7 - 8 weeks old CD1 mice or C57BL/6 (Charles River) were acclimated at least for 48 h and were implanted with the photometry device using a stereotaxic device. The animals were checked daily for moribund or distress condition.

The devices were implanted in the left hemisphere of 7 - 8 weeks old CD1 mice at -3.0 mm AP, +2.0 mm ML, +6.9 mm DV from bregma or C57BL/6 (Charles River) at -1.0 mm AP, +1.0 mm ML, +6.9 mm DV from bregma with at least 48 h of acclimation before surgery. Mice were administered anesthesia using 2 - 3.5% isoflurane. The stereotactic frame and components were sprayed and wiped down with 70% ethanol and placed on a fresh lab mat. A fresh paper towel was placed in the platform for each new animal. All surgical instruments were autoclaved at the start of the day. Surgeons wore clean lab coats, used sterile gloves and masks, and changed gloves for each animal. The animal’s head fur was shaved, and the site scrubbed with Betadine and rinsed with 70% ethanol. The animal was then mounted in the stereotactic frame using ear bars and an incisor clamp. The animal’s eyes were treated with an eye lubricant gel to prevent drying. A 10 mm incision was made with a #10 blade from behind the eyes to the ears and down to the cranium. The scalp was gently spread apart, and the underlying fascia retracted on both sides with Halstead Mosquito forceps. The site was rinsed with fresh saline and swabbed dry. The bregma was used as the stereotactic zero. A 27-gauge needle was stereotactically placed 2.5 mm to the right of
midline on the coronal suture and this site marked with an autoclaved #2 pencil. A small bur hole was made with a variable speed Dremel power tool with a 1.0 mm bur. The probe was inserted into the bur hole with the base of the device fixed to the surface of the skull using super glue. The incision was closed with 4.0 non-absorbable sutures the animal was allowed to recover for 5-10 mins before transfer back to the cage area facility. Mice recovered for days before testing for data transmission rates.

4.10 µCT imaging and MRI.

Detailed information on µCT imaging and MRI parameters are available in supplemental Text ST2.

4.11 Behavioral experiments.

Both control mice (n=5) and mice (n=5) at one-week post implantation received a minimum of 15 mins of habituation to the testing room and experimenters a day before anxiety behavioral experiments. Anxiety experiments were conducted in an open field test (50 cm x 50 cm x 30 cm, a 25 cm x 25 cm square center was defined as “center” in analysis), elevated plus maze (40 cm above the floor) with two opposing open arms (30 cm x 5 cm x 15 cm) and two opposing closed arms (30 cm x 5 cm x 15 cm), and light-dark box (20 cm x 50 cm x 30 cm, dark box 20 cm x 20 cm x 30 cm) each for 6 mins per mice with the arena cleaned with 70% isopropyl in between each test. Mice were recorded with a Logitech webcam and analyzed using Ethovision (XT 10.0, Noldus Information Technology) to track mouse position. During light-dark box experiment, mouse position was tracked only in the light box. Feeding experiments were conducted simultaneously, with each pellet weighed before the 1 h feeding session. Each arena (29.2 cm x
19 cm x 12.7 cm) was cleaned before starting the experiment to minimize urine from affecting the weight of the pellet and feeding behavior of the mice. Each pellet was inspected for urine and weighted again along with any crumbs from the arena. Data from one mouse was rejected due to urine on the food. Force swim tests of implanted mice were conducted in a 5-gallon bucket (diameter = 30 cm) that was cleaned and filled with water. A dual loop antenna with a diameter of 30 cm around the cylindrical container with 1 cm separation at the height of the water level were used to wirelessly power the device at 8 W RF power. Data uplink was established with 5 parallel IR receivers placed 15 cm above the water level. The data communication link was recorded during ~10 mins of full force swim tests.
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APPENDIX B: WIRELESS, BATTERY-FREE, AND FULLY IMPLANTABLE ELECTRICAL NEUROSTIMULATION IN FREELY MOVING RODENTS

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Contributions by Alex Burton: Designed, fabricated, populated, and tested the electronic circuit. Developed a custom communication protocol enabling wireless communication for any microcontrollers with EEPROM without requiring additional components. Implemented control of stimulation amplitude without requiring additional components. Optimized antenna designs with high voltage compliance for controlled biphasic electrical stimulation in rat. Designed Parylene coating that extend device lifetime from previous polyurethane encapsulation methods. Designed alignment tools to guide electrode placement. Designed benchtop tests, in-vivo research experiments, collected in-vivo data, analyzed data, and wrote the paper.
Abstract

Implantable deep brain stimulation (DBS) systems are utilized for clinical treatment of diseases such as Parkinson’s and chronic pain. However, long-term efficacy of DBS is limited and chronic neuroplastic changes and therapeutic mechanisms are not well understood. Fundamental and mechanistic investigation, typically accomplished with small animal models, is difficult because of the need for chronic stimulators that currently either require frequent handling of test subjects to charge battery powered systems or specialized setups to manage tethers that restrict experimental paradigms and compromise insight. To overcome these challenges, we demonstrate a fully implantable, wireless and battery free platform that allows for chronic DBS in rodents with the capability to control stimulation parameters digitally in real time. Devices are able to stimulate over a wide range of frequencies with biphasic pulses and with constant voltage control via low impedance, surface engineered, platinum electrodes. The devices utilize off the shelf components and feature the ability to customize electrodes to enable broad utility and rapid dissemination. Efficacy of the system is demonstrated with readout of stimulation evoked neural activity in-vivo and chronic stimulation of the medial forebrain bundle in freely moving rats to evoke characteristic head motion for over 36 days.

1. Introduction

Wireless battery free investigative tools for targeted neurostimulation of the brain have become important to expand neuromodulation towards freely moving small animal subjects1-5. Continuous wireless power transfer (WPT) to the implants enables ultra-thin platforms that are fully subdermally implantable, which reduces infection risk and more importantly removes the need for tethers to enable experiments in naturalistic environments3. Recent examples include the
first demonstration of neuromodulation in freely flying birds\(^6\) and in multiple socially behaving rodents\(^4\), both of which would be difficult or impossible to achieve with standard tethered approaches. These current demonstrations utilize optogenetic stimulation, which is a powerful tool for exploratory research because of cell-type specific modulation capabilities and has minimal electronic hardware requirements enabling subdermal embodiments that are scalable and feature small footprints. While optogenetics inform translational approaches\(^7\), direct translation is difficult because of the lack of opsin expression in human subjects.

Current clinical neuromodulation therapies, such as deep brain stimulation (DBS) for movement disorders, utilize electrical stimulation. A key challenge to DBS is identifying appropriate stimulus parameters and dosing\(^8\), resulting in up to 50% of DBS patients experiencing side effects\(^9\). The need for parameter optimization and mechanistic insight into DBS therapies motivates the demand for chronic electrical stimulation tools for small animal models such as rodents. In addition to DBS, chronic electrical stimulation is integral to emerging sensory neuroprostheses that restore sight, hearing, and the sense of touch after neurological injury or disease\(^10\). The full stimulus parameter space is rarely explored in neuroprosthetic studies, increasing the reliance on unnatural evoked sensations. With technologies enabling electrical stimulation in freely behaving rodent models, artificial sensory encoding paradigms could be optimized\(^11\).

Current battery powered and tethered methods for chronic stimulation in rodent models complicate studies. Due to the bulky nature of batteries that require frequent charging between experiments and tethered approaches requiring animal care and constant interaction with the test subjects to prevent entanglement\(^12\), current techniques impact subject behaviors\(^1\). A wireless battery free and fully implantable device would alleviate these issues. However, technological
hurdles have, up to this point, prohibited such a device due to requirements for pulse timing, voltage and current modulation, and biphasic stimulation, which are not easily realized in small footprint with commercial components that enable scalable fabrication and rapid dissemination. In this work, we present devices that overcome these current technological challenges by using digitally addressable stimulators that utilize off the shelf components with ultra-small footprint that leverage highly optimized antenna designs and custom one-way communication protocols to enable subdermally implantable wireless, battery free neuromodulators with real-time voltage controlled biphasic stimulation capabilities.

2. Results

2.1 Wireless Deep Brain Stimulation Device

A monolithic design incorporates capabilities of WPT in a thin flexible form factor that enables full subdermal implantation of the DBS as shown in Fig. 1a. The flexible serpentine structure that connects the device body and the injectable stimulation probe allows for easy manipulation of the probe during surgical procedures and provides an interface that facilitates custom probe designs (Fig. 1b and Fig. S1a-c) to control impedance, depth, and spacing of the electrode. The circuit utilizes magnetic resonant coupling (MRC) at 13.56 MHz for WPT by tuning the device antenna with matching impedances to the operating frequency of the primary antenna, which in turn also operates in resonance. The harvesting circuit uses a half bridge rectifier with a Zener diode for over voltage protection. An adjustable low-dropout (LDO) regulator controls input voltage to the microcontroller (µC) which utilizes a feedback loop to adjust stimulation voltage. Biphasic stimulation is delivered by controlling the tri-state (High, Low, High Impedance) of the microcontroller input output (IO) pins. Stimulation voltage is controlled by
regulating operation voltage of the digital system by changing LDO output voltage as schematically shown in Fig. 1c. This method of biphasic stimulation allows for charge balancing of the electrode, improving stimulation responses\textsuperscript{20,21} as well as minimizing tissue damage and corrosion of the electrodes during chronic stimulation\textsuperscript{22,23}.

\textit{Figure B-1. Device overview and summary of operation} a Exploded view illustration of the brain stimulation device (left) and electrode (left). b Photograph of the wireless brain stimulation device with an insert of a micrograph of the probe tip. c Block diagram of circuit function. d Mode of operation during experimental paradigms used in this work. e 3D µCT reconstruction of the implanted device in a rat.

Stimulation parameters are wirelessly transmitted using a custom protocol that is compatible with commercially available power casting systems and features 18 bits to control amplitude, pulse width, period, and duration by modulating radio frequency (RF) power as shown
in Fig. 1d and Fig. S2a-c. Storing these values in the electrically erasable programmable read-only memory (EEPROM) to allow a recall of up to 256 of these parameter spaces. The thin and flexible nature of the device allows conformal adhesion to the skull allowing seamless recovery of the subject after implantation. The device is operational in both magnetic resonant imaging (MRI) and micro-computed tomography (µCT) systems because of careful component selection that features a ferromagnetic free materials. This capability allows for rapid validation of probe and device placement post-surgery as shown in Fig. 1e as well as capabilities to expand experimental paradigms to stimulate electrically while imaging with µCT and MRI.

2.2 Electrode and Stimulation Control

The probe is comprised of multiple thin layers, with a cross sectional dimension of 220 µm x 200 µm (Fig. 2a), minimizing tissue damage during insertion while maintaining mechanical support to increase targeting accuracy, improve stimulation efficacy, and ensure repeatability of experiments. Monolithic fabrication of the probe allows for various electrode designs and to study specific neural pathways. Impedance of the probe is controlled with an engineered high surface roughness of ±400 nm (Fig. S3a) of the platinum electrode material as shown in the scanning electron microscope (SEM) image in Fig. 2b. Impedance characteristics of the electrode are tested before implantation to determine the voltage range needed to elicit neural activation, shown in Fig. 2c. The direct use of µC IO enables high frequency stimulation pulses up to 20 µs and a frequency of 50 kHz. Figure 2d shows corresponding current and voltage traces with an electrode impedance of 10 Kohm at 1 kHz. Current and voltage output is stable under a wide range of loads as shown in Fig. 2e. To minimize footprint, an input voltage adjustment to the µC is used to control stimulation amplitudes. The resulting circuit footprint (20
mm2) is >10x smaller than other wireless electrical stimulation tools and enables designs to be adapted to a variety of animal models. The voltage amplitude (1.5 V – 5.5 V) of the biphasic stimulation is controlled through an analog feedback loop (Fig. 2f) through the adjustable LDO, which is optimized to control the µC input voltage within the voltage range of the µC supply with up to 12-bits of resolution as shown in Fig. 2g and Fig. S3b. Finite element simulation of the field potential and current density of the electrode design with 1 mm spacing are shown in Fig. 2h. Results enable estimation of effective stimulation radius, which, with biphasic pulses of 5 V elicit response up to ~2.3 mm from the electrode sites (Fig. S3c) when considering a current density threshold of >0.97mA/cm².
2.3 Wireless Operation and Mechanical Properties

Fig. 3a shows the dimensional optimization of the secondary antenna that allows mounting of the device on the adult rat skull while maximizing power harvesting capabilities. The secondary antenna utilizes a 6-turn dual layer design (70 µm trace width, 70 µm trace space) that optimizes power delivery while minimizing the secondary antenna cross sectional dimensions (500 µm x 190 µm) to result in the smallest possible device footprint, weighing only 46 mg, which enables faster recovery and easier implantation characteristics. For our experimental paradigm the device is powered in a two-turn primary antenna, circumferentially attached to a 28 cm x 22 cm cage at heights of 4 cm and 8 cm as shown in Fig. 3b, operating at 13.56 MHz. Experimental paradigms are not limited to this antenna configuration. Characterization of harvesting capabilities of the device in several experimental arenas important for both behavioral\(^\text{32}\) and therapeutic\(^\text{33}\) studies are shown in Fig. S4a-d and indicate ample harvested power for device operation in these experimental enclosures. Commercial powering systems with the ability to provide up to 12 W of RF power (Neurolux Inc.) and capability of interfacing with additional peripheral hardware such as levers and buttons to control stimulation delivery can be used for closed-loop control during behavioral experiments that study empathy, attention, feeding, and addiction\(^\text{34–37}\). The secondary antenna design of the fully implantable device is optimized to harvest peak power (18 mW) at 5.5 V in the center of a 28 cm x 22 cm cage as shown in Fig. 3c when powered with 3 W of RF power. This harvesting ability is significantly greater than previous wireless power transfer designs that have been demonstrated with more RF input power and smaller experimental arenas\(^\text{4,15,16}\). The device
consumes an average of 9.35 mW during stimulation epochs with a peak consumption of 29 mW for 0.6 ms during 5V stimulation as shown in Fig. 3d. Lower average power consumption is achieved by optimizing the μC firmware with a 1 MHz clock speed and sleep events that suspend peripheral components of the digital system. Three-dimensional mapping of power is performed at 5 cm and 10 cm heights in a 28 cm x 22 cm cage with 3 W of RF power as shown in Fig. 3e indicating sufficient power throughout the experimental arena to drive stimulation. Investigation in effects of angular misalignments is also conducted in the center of the 28 cm x 22 cm cage with 3W of RF power as shown in Fig. 3f indicating a linear reduction in harvesting capabilities when the device is rotated relative to the primary antenna. Harvesting capabilities during misalignments of the device position and angle respective to the cage antenna indicate stable operation of the device during various naturalistic motions such as rearing. To accommodate these behaviors of the animal the device requires a minimum safety margin of 1.2 which is measured relative to the lowest power availability within the cage. This device provides a safety margin ~2 enabling stable operation in a wide variety of conditions. Cage powers can be adjusted and feature a linear correlation of harvested power as shown in Fig. 3g measured in center of a 28 cm x 22 cm cage. This characterization can be used to estimate power need for larger experimental enclosures. This is demonstrated by operation in increasing cage dimensions (10 cm to 20 cm, radius) (Fig. 3h). Using a safety margin of 1.2 to provide continuous device operation, RF power requirements are calculated for various cage area as shown in Fig. 3i resulting in a maximum arena size of 999 cm² with 10 W of RF power.
Figure B-3. **Device power harvesting, thermal, and mechanical testing.**

- **a** Photograph of the device mounted on a dimensionally accurate 3D printed adult rat skull.
- **b** Photograph of the experimental arena used for in vivo stimulation studies.
- **c** Harvesting power vs. load curve in the center of the arena (22 cm x 28 cm) with RF input power of 3 W.
- **d** Current consumption during operation of the device during a single stimulation pulse.
- **e** Spatially resolved energy harvesting capability at two heights of the miniaturized device in a cage with dimensions of 22 cm x 28 cm and 3 W RF input.
- **f** Harvested power vs. angular misalignment between the device and cage antenna in the center of the arena (22 cm x 28 cm) with RF input power of 3 W.
- **g** Power harvested between RF powers of 2 W and 10 W.
- **h** Power harvested in the center of two turn circular cages with increasing cage radius.
- **i** RF power needed for stable operation with a 20% safety factor in two turn circular cages with increasing cage radius.
- **j** Steady state thermal impact simulation of device under continuous operation in saline.
- **k** Mechanical characteristics of serpentine interconnect.
- **l** Accelerated rate testing of devices encapsulation in PBS.
Steady state simulations of device heating resulting from thermal losses of active and passive components in the circuit are investigated in a phosphate-buffered saline (PBS) solution (36°C) under natural convective heat transfer during continuous operation (>500s). Results shown in Fig. 3j indicate a maximum increase in temperature on the device of <1°C, which is within safety requirements for implanted medical devices by the American Association of Medical Instrumentation39.

Figure 3k shows the mechanical characteristics of the serpentine interconnect that tethers the stimulation probe to the device body. The serpentine structure allows for strains up to 125% enabling easy articulation of the probe during implantation, to target a broad range of brain regions. Device stability is investigated with an accelerated rate test and device lifetime was estimated using Arrhenius scaling40. The devices are submerged in a PBS solution and heated in an oven in a closed container to avoid evaporation. Devices subjected to 90°C failed after 79 days. Devices tested at both 60°C and 43°C are still operational as shown in Fig. 3l and Fig. S5 at the submission of this study, indicating a in vivo lifetime of tens of months. Polyurethane encapsulation which has been used for some devices in this works that do not required extensive chronic stability and has the advantage to avoid cleanroom processing with specialized tools exhibits a lifetime of 34 days in 43°C (Fig S5a), sufficient for work with anesthetized animals and short studies in freely moving subjects. (Fig. S5b).

2.4 Evoked Neurophysiological Activity

Efficacy of the wireless battery free and fully implantable stimulators is demonstrated in an acute experiment to document neurophysiological effects (Fig. S6a). In an anesthetized rat, the bipolar wireless stimulating electrode is stereotaxically placed (Fig. S6b) in vibrissal primary
somatosensory cortex (vS1). A wired recording electrode array (16-channel laminar probe) records activity in the vibrissal primary motor cortex (vM1), to which vS1 is synaptically connected (Fig. 4a). Single biphasic stimulus pulses (3.3 V x 0.2 ms, N = 289) are delivered at 1Hz to vS1 as the response in vM1 is recorded (Fig. 4b). A robust stimulus-evoked potential is recorded in vM1, consisting of both early (5 ms peak) and late (150 ms peak) components that vary across cortical depth (Fig. 4c, demonstrating that wirelessly delivered stimulus pulses are capable of activating cortical circuits.

Figure B-4. Stimulation and recording neurological responses. a Illustration of acute experiment showing location of wireless stimulation site (vibrissal primary somatosensory cortex, vS1) and wired recording site (ipsilateral vibrissal primary motor cortex, vM1) with a segment of vM1 recording during 1-Hz vS1 wireless stimulation (indicated by red vertical lines). b Segment of vM1 recording during 1-Hz vS1 wireless stimulation (indicated by red vertical lines). c Stimulus-triggered average of neural activity
recorded on each of the vM1 laminar probe channels. In both a and c graphs, the two most superficial recordings from the 16-channel array were omitted.

2.5 Chronic Behavioral Effects

Efficacy in freely behaving subjects is demonstrated in chronically implanted rats. The stimulation electrode is stereotaxically placed in the medial forebrain bundle (MFB) and the wireless device body is implanted under the scalp and subsequent sutures close the skin over the device. There are no signs of infection, irritation, or behavioral changes after implantation (1 week). The scalp completely healed over the implant within 3 weeks (Fig. 5a). In this experimental paradigm the stimulation probe is designed to target the (MFB) at the level of the hypothalamus, which contains major dopaminergic pathways. MFB stimulation can activate mesolimbic dopaminergic fibers, producing a pleasing and motivating sensation41. MFB stimulation can also evoke forward locomotion and turning behaviors — the effect sought in this work — putatively due to activating nigrostriatal dopaminergic fibers42–44. Stimuli are manually triggered during sessions in which the rat explores an open field (28 cm x 22 cm). From overhead video, motion of the rat is quantified using a deep learning model (Fig. 5b). Stimulus timing is validated with the illumination of a red LED on the device under the scalp (Fig. 5c), indicating to the operator that the device is active. In addition to scalp healing, locomotor abilities are not impacted by the implant. Impact of the device is measured by tracking exploration in an open field in a manner similar to a naïve control rat (Fig. 5d). The effect sizes of differences in linear and angular head speed between control and implanted rats are small: Hedges’ g = 0.16 and g = 0.05, respectively (Fig. 5e). Finally, the wireless nigrostriatal stimulation is effective in reliably evoking a rapid forward movement (Supplementary Video 1). The evoked movement is tightly locked to stimulus onset, with a mean latency to peak speed of 233 ms. Movement is consistently evoked for over a month following implantation (Fig. 5f). The device eventually failed by post-implant day 44.
Device failure may be due to encapsulation failure or erosion of the electrode interface. Both typical failure mechanisms of implantable electrical stimulation devices and are active fields of research.

Figure B-5. Recovery and behavior of freely moving animal. a Images of rat’s head documenting healing of scalp over the implant. b Example image from overhead video used to quantify the rat’s response to wireless stimulation of the MFB. Superimposed colored circles show the pose estimates for nose, ears, and tail base of the deep learning model used to track movement. c Implanted device LED visible through the scalp on a video frame immediately before stimulation (top) and at stimulation onset (bottom). d Tracked head position within the open field for a control rat and the implanted rat (from 4 x 10 min sessions each). Color of circle at each position indicates head speed. e Distribution of linear (left) and angular (right) head speed for the control and implanted rats. f Mean head speed (± 95% CIM) relative to stimulus onset in 3 testing sessions (blue traces) and relative to random times when animal was at rest in a no-stimulation control session (gray trace).
3. Discussion

The flexible wireless DBS device introduced here is capable of delivering controlled charge balanced stimulation pulses that can be regulated digitally in their delivery frequency (down to 20 µs pulses with up to 25 kHz frequency) and voltage amplitude (1.5 V – 5.5 V). The system can be used in a broad range of chronic experimental paradigms with freely moving subjects in ethologically relevant naturalistic environments, which cannot easily be achieved with battery powered or tethered devices. By using a wireless battery-free approach, we minimize effect on subject mobility (device weight 46 mg) and risks of infection or injuries that arise from group housing. Optimization of the device dimensions facilitates easy subdermal implantation allowing for fast recovery times and no change to animal behavior compared to naïve control animals. The technologies presented here further enable a wide range of customization, adoption of various electrode designs, such as the ones presented here or other flexible electrode technologies, that can be exchanged to target brain regions of interest. Additionally, real-time wireless control over stimulation parameters in cage dimensions of up to 999 cm² enables a wide range of experimental paradigms. Small size and minimal footprint of the implants and the use of ferromagnetic free off-the-shelf components enables compatibility with noninvasive 3D imaging of subjects and facilitates the possibility of broad dissemination with existing scaled manufacturing technologies. Successful chronic experiments and scalable technologies demonstrated in this work suggests potential for widespread adoption in neuroscience research and enables future studies to explore chronic electrode performance, stimulation induced neural plasticity and chronic closed-loop behavioral studies in freely moving subjects. The technology may also serve as a platform to enable wireless and battery free operation for features such as neural recording and muscle interfaces.
4. Materials and methods

4.1 Flexible Circuit Fabrication

Copper traces were defined on pyralux (AP8535R; constituent layers: 17.5-µm copper, 75-µm polyimide, and 17.5-µm copper) using a UV (355-nm) laser ablation system (LPKF; Protolaser U4). The flexible circuits were cleaned in stainless steel flux (Superior Flux and Manufacturing Company; Superior #71) for 2 minutes in an ultrasonic cleaner (Vevor; Commercial Ultrasonic Cleaner 2L) and rinsing with deionized (DI) water. Via connections were established manually with copper wire (25 µm) and low-temperature solder (Chip Quik; TS391LT). Combinations of 0201 capacitors (108 pF) were used to tune the power harvesting antenna. A half-bridge rectifier was built with low-capacitance Schottky diodes (Skyworks) and three 0201 capacitors (2.2 uF). Zener diode (Comchip Technology Corporation; 5.6 V) provided overvoltage protection to limit supply voltage to the adjustable LDO (Maxim Integrated, MAX38902C) used to stabilize the input voltage to the µC (Atmel; ATtiny 84A). A custom programmer board using Arduino as ISP was used to program the µC before mounting on the circuit. The µC provided controls over visual indication through a red LED with a 3.3 kΩ current limiting resistor, controlled timing of electrical stimulation, and LDO output voltage. Components were reflowed with a hot air gun 350 °C using low-temperature solder (Chip Quik; TS391LT). Devices were tested with a reflection bridge (Siglent; SSA 3032X; RB3X20) and additional tuning capacitors are added to provide the lowest voltage standing wave ratio at 13.56 MHz. Silver particle-filled epoxy (Model 8331, MG Chemicals Inc.) established electrical connection to the probe and cured at 65 °C for 30 minutes. The connection was mechanically joined using UV curable glue (Damn Good; 20910DGFL) and cured under a UV lamp (24 W, 10min). A tungsten foil (Alfa Aesar, CAS# 7440-33-7) was defined
through laser ablation (LPKF; Protolaser U4) and mounted to the back of the probe using cyanoacrylate as structural support.”

4.2 Stimulation Electrode Fabrication

Figure S7 presents a schematic illustration for the fabrication and layout of the stimulation electrode. The first step involves the removal of copper layer from flexible copper-clad polyimide film (AP9111R, DuPont), yielding 25 µm thick polyimide with surface roughness of ±400 nm. This substrate served as a template to introduce the surface roughness on subsequent thin film metal layers. Photolithography and Lift-off process, followed by electron beam evaporation (Cr/Au/Ti/Pt, 5/100/5/100 nm), defined both stimulating metal electrode and interconnects. Coating of polyimide (PI2545, HD MicroSystems; 2 µm in thickness) served as the passivation layer and opened the contact pad by reactive ion etching (O2, 100 mTorr, 100W, 20 sccm, 10 min) and exposed the platinum (area of 130 µm x 130 µm) stimulation electrode. A 75 µm thick polyimide film served as the supporting substrate for short electrodes (below 4 mm) and an addition of a tungsten shuttle (50 um thickness) served as a stiffener to enable high accuracy targeting, both supporting substrates were attached to the electrode film with adhesive layer (Polydimethylsiloxane). Laser cutting process (ProtoLaser U4, LPKF) completed the formation of the electrode layout.

4.3 Electrode Characterization

The electrical behavior of the microelectrodes was studied in Dulbecco’s phosphate-buffered saline (PBS) solution (14190-136, Gibco, Life Technologies). Three-electrode electrochemical cell including a stimulating electrode, Pt wire, and Ag/AgCl electrode (MF-2052,
BAS), working, counter, and reference electrodes, respectively, were immersed in PBS. The electrochemical impedance spectroscope (Autolab PGSTAT128N) measured the impedance of the working electrode at frequencies ranged from 0.1 Hz to 100 kHz under an applied voltage input of 5mV. Simulation output of the wireless and battery free device was characterized by programming of commands through RF modulation that is controlled by an Arduino Nano (Atmel; ATmega328) that communicates over a serial port to a computer. Device were wirelessly programed through RF modulation with 3 pulse widths with an amplitude of 5V. The current and voltage was recorded with a source measure unit (Keithley, Model 2450 SourceMeter®) with a 10 kΩ load. Device with 5V stimulation and current was measured (LowPowerLab; CurrentRanger) with various resistive loads. Amplitude of stimulation was wirelessly programmed and measured with an oscilloscope (Siglent; SDS 1202X-E) with a 10 kΩ load.

4.4 Encapsulation

Devices were rinsed with IPA for 10 minutes and air dried. UV curable glue (Damn Good; 20910DGFL) was added over components and finally cured under a UV lamp (24 W, 10min) and degassed in an oven (100 °C, 5 min) to add mechanical protection to the solder interface of the surface mounted components. Conformal coating was achieved by parylene encapsulation, the electrode tip was covered with polyimide sheets on either side of the probe to protect the electrode surface from coating and the seams were held together using parafilm. Devices were suspended along a wire and encapsulated using the Parylene P6 coating system (Diener electronic GmbH, Germany) with 2 coating runs each using 5.0 g of Parylene-C dimer for a total thickness of ~18 µm, covering the entire device surface conformally with Parylene C proving a moisture barrier and a biocompatible biointerface. Encapsulation thickness was calculated from measurements using a
profilometer (Tencor P15, KLA) and subsequently controlled using Parylene-C dimer weights (Fig. S8). For polyurethane coated devices, after covering the probe tips and hanging them on a wire, were sprayed with premium polyurethane conformal coating (4223F, MG Chemicals Inc.) and cured at 90°C for at least 12 hours. The devices were finally dip coated with PDMS (SYLGARD™ 184 Silicone Elastomer kit) and the excess was removed using a syringe and cured at 80 °C for 10 min.

4.5 Power Harvesting Characteristics

Voltage and power harvesting characteristics were collected (Aneng; AN8008) using a load resistor in the center of a 28 cm x 22 cm arena powered with a dual loop antenna with 3 W of RF power. A shunt resistor (1.6 kΩ) was used to match the system load during harvested power measurements in 3D maps at heights of 6 and 10 cm and angular misalignment between the cage antenna and device antenna using an oscilloscope (Siglent; SDS 1202X-E) in a 28 cm x 22 cm arena with 3W of RF power. Harvested power measured (Siglent; SDS 1202X-E) in the center of the cage with RF power ranging from 2 W to 10 W. Current consumption was recorded with a modified current meter (LowPowerLab; CurrentRanger) and acquired using an oscilloscope (Siglent; SDS 1202X-E). Harvested power was measured (Siglent; SDS 1202X-E) using a shunt resistor (1.6 kΩ) in the center of dual loop antenna with diameters of 10 cm to 20 cm with varying powers and linearly fit in between powers of 0 W to 16 W. Average power consumption combined with a safety factor of 20% was used to determine required delivered RF power for stable operation in the center of the varying sizes of circular cages.
4.6 Electrical and Thermal Simulation

Finite-element simulation of field potentials and current density was done in COMSOL ® Multiphysics with a stimulation of 5 V. Accurate probe dimensions and material properties for dielectric, electrical conductivity, and relative permittivity were used for each material: polyimide (6.66 S/m, 3), platinum (9.4e6 S/m, 0.0039), and saline (1.3 S/m, 75) and analyzed during the first biphasic stimulation pulse.

Finite-element simulation of heat transfer in solids and fluids after 1000 seconds of continuous operation of the brain stimulation device was performed in COMSOL ® Multiphysics. Integrated components, copper traces, and polyimide were accurately modeled and natural convection in saline with an initial temperature of 36°C to mimic average temperature in rats was used as starting parameters. Heating power applied to components were: μC 8.6 mW; LDO 5 mW; rectifier 10 mW; LED 0.5 mW; LED Resistor 0.5 mW. Thermal conductivity, heat capacity, and density were: component mold compound (0.5 W m−1 K−1, 1000 J kg−1 K−1, and 1350 kg m−3), inner dies (130 W m−1 K−1, 678 J kg−1 K−1, and 2320 kg m−3), copper (400 W m−1 K−1, 385 J kg−1 K−1, and 8900 kg m−3), polyimide (0.2 W m−1 K−1, 1100 J kg−1 K−1, and 1470 kg m−1), and saline (0.6 W m−1 K−1, 4180 J kg−1 K−1, and 1000 kg m−3).

4.7 Mechanical Testing

The device was mounted on a scale (Mettler Toledo; AB104-S). The probe was fixed to a custom 3D printed slider to measure the displacement of the device as it was stretched using a digital caliper (electronic digital caliper). The serpentine structure was stretched with 1 mm increments.
4.8 Encapsulation Testing

Three encapsulated devices (PDMS, Polyeurethane+PDMS, and Parylene-C+PDMS) were submerged in a 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride (SIGMA, P4417) in sealed glass vials at 43 °C and recorded daily to visually check operation using an indictor LED. This test was also conducted with devices coated with Parylene-C and PDMS in PBS temperatures of 60°C and 90°C and also checked daily for device operation.

4.9 Acute In Vivo Testing

Both the acute and chronic experiments were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. The study used three male Sprague Dawley rats (Crl:SD, 275 to 325 g): one for the acute experiment, one chronically implanted, and one unimplanted control. For the acute experiment, the rat was anesthetized with intraperitoneal injection of ketamine (60 mg/kg) and dexmedetomidine (0.25 mg/kg) and placed in a stereotaxic frame. Throughout the procedure, depth of anesthesia was monitored by respiratory rate and pedal reflex and maintained at a surgical plane with additional injections of ketamine as needed. A large craniotomy was performed to expose the right vS1 and vM1. A 16-channel laminar probe with 50 μm electrode diameters (Microprobes for Life Science) was placed in vM1 to recorded stimulus-evoked activity. The recordings were referenced to a remotely placed subdural wire, with a 00-90 skull screw placed in the frontal bone serving as ground. The wireless stimulating electrode was placed in vS1. Single electrical pulses (3.3 V x 0.2 ms) were delivered to vS1 at 1 Hz while recording from the vM1 array using an Intan RHS system (Intan Technologies).
4.10 Chronic Implantation

The rat was anesthetized with 5% isoflurane in oxygen and placed in a stereotaxic frame. Buprenorphine SR (1.2 mg/kg) was administered subcutaneously for long-acting analgesia. Throughout the procedure, depth of anesthesia was monitored by respiratory rate and pedal reflex and maintained at a surgical plane with 1.5 to 2.5% isoflurane. A 1.5-mm diameter craniotomy was centered on a point 2.8 mm posterior to bregma and 1.7 mm lateral to midline. A small slit was made in the dura. Two 00-90 skull screws were implanted anterior and posterior of the craniotomy. The device was mounted on a manual micromanipulator using a toothless micro alligator clip attached to the base of the probe. The tip of the probe was slowly lowered to a depth of 8 mm below the dura, into the MFB at the level of the lateral hypothalamus. While lowering, the device antenna was tucked underneath the scalp. After lowering, a small amount of acrylic dental cement was applied to bond the probe to the skull screws. After removing the clip, more acrylic was applied to cover the probe and screws entirely, while ensuring that no acrylic came into contact with the antenna or other aspects of the device. The scalp was then sutured over the implant. After one week recover, sutures were removed, and experiments began.

4.11 Behavioral Experiments and Analysis

Experimental sessions consisted of placing the chronically implanted rat or the control rat in the wireless stimulating open field and using videography to capture the animal’s pose during exploration. In the case of the implanted rat, stimulation was occasionally triggered manually by a battery-powered switch. To avoid an acoustic startle response, care was taken to ensure there was no auditory indication of the electrical stimulus. Stimulation was delivered during periods of immobility to more easily distinguish stimulus-evoked movement from volitional movement.
Overhead video was recorded in 1080p HD at 30 frames/s. Data analysis began by extracting pose and stimulus timing information from the videos. Pose estimation was performed using DeepLabCut\textsuperscript{48}. A convolutional neural network was trained on a GPU-accelerated virtual machine in Google Colaboratory to track the position of the nose, ears, and tail base. Stimulus onset was determined from the illumination status of the implanted LED on each frame using an image processing script in MATLAB. Then, the speed of each tracked body part was estimated using the finite difference approximation after removing pose estimates with likelihood scores less than 0.995. Finally, the mean evoked speed across stimuli and 95% confidence intervals on the mean (CIM) were computed in a 2.5-s window around stimulus onset.

### 4.12 μ-CT Imaging

At the conclusion of the chronic experiment, the rat was euthanized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with heparinized saline followed by 10% neutral buffered formalin (NBF). The head was soaked in NBF for 24 h before removing soft tissues around the skull and loading it in a 50 ml centrifuge tube for imaging. The whole head was scanned using 90 kVp with copper filter at 14.6 μm isotropic resolution (μCT45 scanner, Scanco Medical). The μ-CT DICOM stack was imported into ImageJ and all dimensions were deduced to 25% of its original size. Image stack contrast and brightness was automatically adjusted to the background. The plugin volume viewer was used to view the CT image as a volume with a 2D gradient thermal LUT color overlay.
References


APPENDIX C: OSSEOSURFACE ELECTRONICS – THIN, WIRELESS, BATTERY-FREE AND MULTIMODAL MUSCULOSKELETAL BIOINTERFACES

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Contributions by Alex Burton: Designed, fabricated, populated, and tested the electronic circuit. Implemented custom communication schemes to enable higher communication speeds while using a NFC compatible IC to record rat gait movement. Optimized antenna designs to balance effective power harvesting and communication capabilities. Improved power consumption for this device operating at low voltages. Designed benchtop tests, in-vivo research experiments, collected in-vivo data, analyzed data, and wrote the paper.
1. Introduction

Continuous recording of bio-signals with high fidelity has been widely recognized to play a key role in modern exploratory research, diagnostics and therapeutics. Specifically, with the emergence of computational tools such as neural networks, artificial intelligence and machine learning that can help to analyze large datasets, continuous high-quality data streams will enable the development of diagnostics and therapeutics that will result in significantly improved patient outcomes. However, current biosensing platforms with clinically relevant data streams rarely extend recording beyond short time periods. This is due to inadequate powering solutions, such as bulky electrochemical power supplies and biointerfaces that degrade rapidly requiring intervention by users or health care providers, thus limiting the utility for exploratory, screening, diagnostic and therapeutic applications.

Recent integration of high-performance silicon-based devices and emerging soft electronics yields numerous subdermally-implantable neuromodulation systems that interface intimately with the central and peripheral nervous system. This technological platform enables wireless supply of power and communication for highly miniaturized implants, allowing for the creation of interfaces to organs that are currently understudied due to the lack of suitable tools. One such area is the musculoskeletal system where wireless, battery-free interfaces are critical to evolve drug discovery, diagnostic and therapeutic capabilities. Just one example of clinical need are fragility fractures associated with osteopenia and osteoporosis that account for more hospital bed days than myocardial infarction, breast cancer or prostate cancer. These fractures cause high mortality and long-term disability with health care cost over $25 billion per year by 2025. Thus, technologies that directly and continuously monitor bone quality, and enable exploratory research...
towards advanced therapeutics in a form factor that enables broad dissemination and a convenient study platform will considerably improve patient quality of life and reduce healthcare costs.

Here we introduce a device platform that uses intimate integration with the osseousurface, the surface of the bone, to enable chronic monitoring of the musculoskeletal system in small and large animal models and lays the foundation for clinical diagnostic tools that can be operated using broadly available near field communication (NFC) standard in deep tissue. The wireless, battery-free, and fully-implantable devices, named osseousurface electronics, can be attached to the surface of bones during orthopedic surgeries and form a chronic interface with bone tissues to directly record a multitude of physiological and biophysical signals critical for the assessment of musculoskeletal health and deliver stimulation in real time (Figure 1a), providing a powerful point-of-care platform to facilitate rehabilitation and to manage musculoskeletal diseases.
Figure C-1. Osseosurface electronics: concept, device architecture, and implementation strategies. 

a) Illustration of osseosurface electronic systems that are permanently bonded to the bone and operate wirelessly to continuously monitor biophysical signals such as bone strain, local temperature, and to deliver optical stimulation to the bone and surrounding tissues. 
b) Photograph of an osseosurface device designed studies in large animal models. 
c) Layered makeup of the osseosurface system and its constituent layers. Inset features a close-up view of the multifunctional biointerface comprised of a metal foil strain gauge, an NTC thermistor and a µ-ILED. 
d) Photograph of an osseosurface device conformally attached on the surface of a sheep humerus. 
e) Functional block diagram of osseosurface electronics comprised of an external NFC reader that provides power and facilitates wireless communication, and an implanted system that contains active power management, operational control, analog front-end (AFE) and biointerface. 
f) Photograph of a rat (2 weeks after surgery) implanted with an osseosurface device where the main electronics reside on the back while the biointerface is routed and attached on the left femur.

2. Results

2.1 Device design.

The creation of osseosurface electronics requires several technical innovations that differ from epidermal electronics such as device footprint and mechanical properties suitable for direct lamination onto the bone surface to minimize mechanical mismatch with the surrounding tissues,
and electromagnetic design allowing for direct readout through thick tissues with portable devices to enable smart therapeutics. Special attention to mechanical design of interconnects is required to enable chronic stability of the interconnect and minimize mechanical impact on the targets sensing region to avoid introduction of additional strain. Figure 1b shows a device that meets these design criteria (2.5 cm x 1.5 cm, ~170 mg) and enables direct, conformal lamination to the curved osseousurface with minimal impact on the surrounding tissues. The device features mechanically isolated biointerfaces that are capable of measuring deformation of the bone with microstrain accuracy, high fidelity thermography with mili-Kelvin resolution and photonic stimulation capabilities. The system features a hybrid integration of mechanical compliant flexible substrate with high-performance analog and digital functionalities provided by miniaturized off-the-shelf components (Supplementary Figure 1 and Supplementary Figure 2) arranged in a configuration that enables conformality and reliable operation when applied to the curvilinear surface of the bone, as depicted by the layered device makeup shown in Figure 1c. The inset of Figure 1c highlights the multifunctional biointerface comprised of a metal-foil strain gauge, a negative temperature coefficient (NTC) thermistor and a microscale inorganic light emitting diode (µILED), enabling simultaneous recording of local biophysical signals and on-demand delivery of exogenous stimulation. The device geometry is highly adaptable to accommodate implementation scenario and anatomic structure. Figure 1d shows a device variant applied to a sheep humerus, a testbed to gain insight on capabilities for use as a diagnostic tool after recovery from surgery. We also introduce devices designs for exploratory research in small animal models.
Figure C-2. System characteristics of osseous-electronic systems. a-c Device for large animal models: Device photograph (a). Harvested power and voltage as functions of electrical load (b). Spatial distribution of harvested power using a hand-held primary antenna at a load of $300 \, \Omega$ (c). d-f Device for small animal models: Device photograph (d). Harvested power and voltage as functions of electrical load (e). Spatial distribution of harvested power using a 45 cm x 12 cm primary antenna measured with a load of $\sim900 \, \Omega$ (f). g Power consumption of the device operating at different modes: I. temperature sensing; II. temperature and strain sensing; and III. temperature and strain sensing, and optical stimulation. Modes II and III are represented by green and red dashed lines in b and e that indicate the value of electrical load and power consumption. h Data rate of wireless communication for a large animal device (immersed in PBS solution) read and powered by a hand-held primary antenna as a function of antenna to device distance. Inset, 3D rendering of the experimental setup. i Demonstration of long-term data recording, for a small animal device (immersed in PBS solution), measured with the 45 cm x 12 cm primary antenna on a custom-built metal-free rat treadmill with back-and-forth motion at a speed of 25 cm s$^{-1}$. Wireless results are benchmarked against environmental temperature recorded by a thermometer (red data line) placed in close proximity. Inset, 3D rendering of the experimental setup.

Figure 1e describes the electrical working principle of the system that enables wireless, battery-free operation in a form factor suitable for full implantation that can adopt various NFC chipsets (Supplementary Figure 3). Near-field magnetic resonant coupling (13.56 MHz, specific absorption rate (SAR) < 20 mW kg$^{-1}$)$^{12}$ between an external primary loop antenna (primary
antenna) and the on-board loop antenna (secondary antenna) enables, for the first time, reliable radio frequency (RF) power harvesting through thick tissues up to 11.5 cm with hardware that is compatible with NFC protocols widely available in portable devices\textsuperscript{17}. Optimized harvesting electronics matched to digital and analog electronics provide power to an NFC system-on-chip (SoC) that enables operational control through a microcontroller (μC) and NFC transponder in a compact package (4 mm x 4 mm), and analog front ends (AFE) comprised of passive filters and instrumentation amplifiers that read out thermographic and strain sensors. Design of the sensing circuits is facilitated by circuit simulation (details in the Methods section), yielding a strain sensing AFE that consumes 3.24 mW with a sensitivity of 0.194 mV με\textsuperscript{-1}, and a thermography AFE that consumes < 0.06 mW with a sensitivity of 98.9 mV °C\textsuperscript{-1} (Supplementary Figure 4 and Supplementary Figure 5). Digital engineering and manufacturing enable rapid development and deployment of osseosurface electronics in form factors suitable for a variety of operational conditions. Figure 1f shows an example of a system variant that enables operation in a freely moving small animal model without tethers or other externalized elements, which allow advanced exploratory studies. The ultrathin mechanics and low displacement volume (< 0.2 cm\textsuperscript{3}) of the device enable rapid recovery of the subject from surgical placement and high-fidelity behavioral studies while collecting bioinformation in real time which is not possible with existing wire bound technologies.

2.2 System characteristics.

Consistent operation of osseosurface electronics relies on robust wireless power transfer through tissues for large animal models and for therapeutic applications. In the case of small animal models for exploratory research free motion in a variety of test arenas is required. Both scenarios
required robust power transfer and efficient wireless power transfer via magnetic resonant coupling that can be boosted by adopting an secondary antenna with high quality factor, in our case antennas with high inductance and low impedance\textsuperscript{18}. Figure 2a displays a large animal model device with optimization through iterative imperial electromagnetic designs (Supplementary Figure 6) of the secondary antenna using \( \approx 185 \) pF to match the reactance of the antenna inductance of \( \approx 745 \) nH (3 turns, \( 600 \mu m \) wide, \( 60 \mu m \) spacing) at 13.56 MHz allowing for low trace impedance (\( \approx 30 \) m\( \Omega \))\textsuperscript{19}, for operational voltages of 2.5 V. As shown by the power harvesting characteristics (Figure 2b and Supplementary Figure 7) measured at the center of a handheld primary antenna (diameter \( \approx 20 \) cm), the maximum values of harvested power (\( \approx 14 \) mW) and rectified voltage (\( \approx 2.1 \) V) support operation at an electrical load of \( \approx 300 \) \( \Omega \). Circular symmetry of the handheld primary antenna (2 - 8 W of RF power) results in minimal spatial variation in harvested power (Figure 2c and Supplementary Figure 8) in close proximity to the antenna and at physiologically relevant distance (5 cm) that corresponds to the depth of implantation on a sheep or human humerus.

To provide power to freely moving small animals, critical for exploratory research in large test arenas (treadmill cage, 45 cm x 12 cm; home cage, 26 cm x 33 cm), devices designed for rat models (Figure 2d) require an enlarged secondary antenna (3.5 cm x 2.5 cm) and device layout that features serpentine interconnects (\( \approx 11 \) cm at full extension) to route the biointerface from the back of the subjects which houses the electronics and antenna section of the device to the limb that is the sensing target in our experiments. Optimization through iterative imperial electromagnetic designs (Supplementary Figure 6 and Supplementary Figure 7) of the secondary antenna using \( \approx 485 \) pF to match the reactance of the antenna inductance of \( \approx 284 \) nH (2 turns, \( 600 \mu m \) wide, \( 60 \mu m \) spacing) at 13.56 MHz allowing for low trace impedance (\( \approx 3.3 \) m\( \Omega \))\textsuperscript{19}, enables harvesting performance of a rectified voltage of \( \approx 2.2 \) V at a load of 300 \( \Omega \) (16.13 mW) at the center of the 45
cm x 12 cm cage, providing a margin of 0.4 V to enable constant system voltage of 1.8 V throughout all experimental conditions (Figure 2e). The spatial distribution (Figure 2f) of the harvested power in the 45 cm x 12 cm cage exhibits sufficient power for stable device operation with primary antenna winding heights at 3 cm and 6 cm chosen to match device body implantation location in the freely moving subject (Supplementary Figure 9). Similar results are obtained for the 26 cm x 33 cm primary antenna, revealing a rectified voltage of ~2.1 V at a load of 300 Ω (14.7 mW) at the cage center and sufficient power at physiologically relevant heights (Supplementary Figure 10a-b). A comparison between the power harvesting capability and the power consumption of the biointerface provides a practical guide for feasible in-vivo operation modes. Figure 2g shows system power consumption, specifically ~3.24 mW in sensing mode, and ~10.26 mW as optical stimulation is activated, which represents ~60% and ~50% of the harvested power at the corresponding electrical loads, i.e., 900 Ω and 300 Ω, respectively (Figure 2e). Considering the dependence of power harvesting capabilities on tilt angle and bending curvature radius (Supplementary Figure 10c-e), stable and sufficient power can be guaranteed as long as the tilt angle is below 50° and curvature radius is above ~1 cm.

Wireless communication is crucial for implantable devices and often limits device operation in thick tissues. Benchtop experiments reflecting this scenario reveal that the device designed for deep tissue implantation immersed in PBS solution supports a nearly constant data rate of ~46 points per minute with a reading distance up to 7.5 cm from the handheld primary antenna (Figure 2h) enabling operation as a diagnostic device in large animal models and human subjects. Similar tests designed for use in rodent models reveal uniform data rate in test arenas for rats such as the treadmill cage and home cage (Supplementary Figure 11). Long-term data recording (Figure 2i) tests performed in the biologically relevant settings with a moving
osseosurface electronic devices, (details in Methods section) results in stable communication over extended periods of time (42 hours, no limitation in operation time) where the recorded temperature profile matches that of environment temperature measured by a spatially separate thermometer. Further studies of chronic device encapsulation (18 µm Parylene-C) and device operation are conducted via an accelerated rate test in 90 °C, 60 °C and 40 °C PBS bath to enable device lifetime estimation (205 days at 37 °C) via Arrhenius scaling21 (Supplementary Figure 12).

2.3 Biointerface characterization.

Characterization of the multimodal biointerface is performed with benchtop experiments that reflect the in vivo environment. Wireless strain sensors exhibit sensing performance on par with the gold standard wired strain measurement systems in typical physiologically relevant range (0 - 1200 µε)22, as confirmed by ramping and cyclic loading tests performed on a sheep bone specimen (Figure 3a-c, details in Methods section), with an estimated sensitivity of ~3.5 ADC µε-1 and a resolution of ~14.3 µε with a sampling rate up to 87 Hz. Cyclic loading tests up to 10 Hz show recording capabilities that surpass gold standard acquisition systems for recording bone strain (Supplementary Figure 13a-c). Simultaneous recording of temperature and strain with optogenetic stimulation is shown in Supplementary Figure 13d. Repeatable, hysteresis-free response is obtained upon progressively increasing loads (Figure 3b), indicating that a stable bond is formed between the strain gauge and the bone specimen. Reliability of the wireless strain sensor is further demonstrated by cyclic loading with sinusoidal (Figure 3c), square and triangle (Supplementary Figure 14) wave forms.
Current implantable biointerfaces for the musculoskeletal system are typically limited to wired strain sensing\textsuperscript{23-27}, however our device architecture offers the multimodal integration of stimulation capabilities currently only available in tethered embodiments\textsuperscript{28-32}. To demonstrate the feasibility of optogenetic stimulation capability in the periphery\textsuperscript{33} on an osseousurface electronic
platform, we use miniaturized individually addressable μ-ILEDs to deliver stimulation to the bone and the surrounding tissue. The bone-tissue interface provides a unique platform capable of versatile optical coupling modes tailored for various application scenarios, such as phototherapeutic stimulation for bone regeneration\(^{28}\) and optogenetic activation of muscular contraction\(^{32}\). Figure 3d demonstrates this design flexibility with devices that are capable of illuminating the soft tissue side (left), bone side (middle), and multimodal stimulation (right). Typical operation for optical stimulation covers a parameter space of 5 - 20 Hz for applications such as sustained tetanic contraction\(^{31,32}\), which is achieved on our platform by utilizing a μC for precisely controlled timing as demonstrated in Figure 3e. The μ-ILEDs used to create an ultraminiaturized form factor (240 μm x 240 μm x 100 μm) and high energy efficiency (> 20%, Figure 3f), ensure minimal thermal impact to the tissues\(^{34}\).

Continuous monitoring of the local temperature can simultaneously be achieved on our platform with a miniaturized (0201, 0.6 mm x 0.3 mm) NTC thermistor that is integrated monolithically on the biointerface. The wireless temperature sensor exhibits linear response in physiologically relevant temperature ranges (33 - 41 °C) with a sensitivity of ~1920 ADC K\(^{-1}\) and a resolution of 10 mK (Figure 3g-h, details in Methods section), and can be used to monitor physiological events or to directly assess thermal impact of the μ-ILED, enabling closed loop control for phototherapeutic and stimulation applications requiring deep tissue penetration. Figure 3i presents the thermal impact of the μ-ILED operating at increasing intensities (30 - 170 mW mm\(^{-2}\)) and duty cycles (5 - 80%) in a physiologically relevant environment mapping the parameter space for optogenetic stimulation and phototherapy. Experimental measurements are in agreement with FEA simulation (Supplementary Figure 15)\(^{34}\) enabling computational design of the interface. Irradiation that can be delivered by our platform (e.g. 30 mW mm\(^{-2}\)) is well beyond the intensity
used for optogenetic activation of skeletal muscles\textsuperscript{31,32} and evokes only a 0.29 °C temperature increase at a duty cycle of 80%. This suggests operation over a wide range of conditions without thermal activation of neuronal function.

2.4 Mechanical characterization

Subdermal implantation in small animal models involves placement in highly mobile areas which require a compact formfactor with mechanical design strategies that can withstand repeated strain cycles without interfering with biosensor readouts. This is evident when studying the micro-CT scan (Figure 4a) of a rat with an implanted osseosurface electronic device, where the device body is anchored subdermally around the lumbar vertebra and connection to the biointerface, which is located on the left femur, is accomplished via self-similar serpentine interconnects\textsuperscript{35}. The durability of the serpentine interconnect is critically important for reliable in-vivo operation. FEA simulation guided design enables reliable performance under repeated strains of over 250% while maintaining a maximum strain of ~0.7% in the copper traces which is below 1%, the failure strain of copper (Figure 4b)\textsuperscript{35}. Chronic electro-mechanical stability is confirmed by cyclic straining to 250% for 10,000 cycles, with negligible change in conductivity (Figure 4c). Critical to the stability of sensor readings during behavior is the mechanical isolation of the stretchable interconnect and the strain sensor, this includes introduction of strain to the bone during deformation of the serpentine interconnect. The biointerface is mechanically isolated by the serpentine interconnects designed to transfer minimal strain during deformation to the bone and the strain sensor biointerface, as confirmed by FEA in Figure 4d (Supplementary Figure 16) showing that stretching the serpentine by 250% does not influence the sensitivity or accuracy of the strain gauge (Figure 4e-f and Supplementary Figure 17, details in Method section). Further evaluation of serpentine
Electro-mechanics are performed with bench-top experiments using a servo-hydraulic materials testing system (MTS) indicating stable operations of recording capabilities under multiple cycles of high strains (Supplementary Figure 18) above physiological strains as means to evaluate robustness of the approach. Both benchtop electro-mechanical testing of the monolithic serpentine structure with FEA simulations of the serpentine shows solid performance in strain isolation enabling flexible sensor placement to distal regions with minimal effect of sensor readouts.

Figure C-4. Mechanical design and characterization of osseousurface electronic devices designed for rodents. a Micro-CT scan of a rat implanted with an osseousurface electronic device. The device is highlighted in yellow. b Photograph of the self-similar serpentine interconnects stretched to 250% (upper) and FEA strain profile of the serpentine interconnects stretched to 250% (lower). c Resistance of the interconnects at 0 and 250% strain during cyclic stretching to 250% for 10,000 cycles. d FEA of the biointerface when the bone is compressed by 1000 µε while the serpentine interconnects are in two states: i. relaxed (0% strain); ii. stretched (250% strain). e Wirelessly recorded ADC values when the strain gauge is cyclically loaded and unloaded while the serpentine interconnect is strained to 250%, the inset shows the test setup with 250% strain applied. f Wirelessly recorded ADC values when the strain gauge is cyclically loaded and unloaded after the serpentine interconnect has been strained for 0 cycle, 5000 cycles and 10000 cycles.

2.5 In-vivo studies in rodents.

The subdermally implantable form factor and wireless, battery-free operation enable an in-vivo multimodal bi-directional interface with the musculoskeletal system without compromising the free motion of subjects in various test arenas (Figure 5). The implantation of osseousurface
electronics in rats involves a skin incision on the back of the subject, device placement into the subcutaneous space, subcutaneously tunneling the biointerface to the limb, attaching the biointerface to the femur with cyanoacrylate, and closing the skin with resorbable sutures (Details provided in the Methods section and Supplementary Figure 19, following the University of Arizona Institutional Animal Care and Use Committee approved protocol (19-572 IACUC). Devices are tracked and monitored after surgery to gain insight into failure mechanisms. Majority of device failures examined after explanation indicate defects in the Parylene-C encapsulation induced by surgical tools (Supplementary Table 1). Mitigation of these issues in later experiments utilized sutures to manipulate and position the biosensor, resulting in lower failure rates. The optoelectronic interface attached on the bone surface allows for direct optical stimulation of skeletal muscles in freely-moving subjects, as shown in Figure 5a where illumination of the muscle is visually validated, highlighting a systematic advantage over traditional transdermal light sources\textsuperscript{30} or optical fiber-based techniques\textsuperscript{29} which are challenging to implement in highly mobile areas. Light delivery can be programmed to control frequency and duty cycle of stimulation each with 16-bit precision. Chronic functionality is demonstrated with in frame analysis of tracked red pixel intensity over time (Supplementary Figure 20) from video recordings 22 days post implantation (Supplementary Video 1). In-vivo device operation of temperature sensing capabilities is tested over extended time periods demonstrated by a 10-minute moving window of thermographic recording that show recovery of the animal post-surgery for over 11 hours in the home cage (Blue) with a standard deviation within the 10-minute moving window (pink) (Figure 5b). Following the surgical implantation of the device a dip in local body temperature during the first half hour of recovery after implantation is observed which is an expected hypothermic response to isoflurane anesthesia\textsuperscript{36} and transfer from a warmed surgical table to a cooler recovery.
area. Following recovery from anesthesia the limb temperature remained within the published range of normal body temperature for male Sprague Dawley rats. Strain profile recorded wirelessly in a custom treadmill arena with the 45 cm x 12 cm primary antenna reveals characteristic loading and unloading phases with absolute strain values comparable to literature values for the rat femur obtained by tethered sensors. Time synced wireless recording of strain and temperature are compared with video recording of the animal during walking periods after a week of recovery (Supplementary Video 2). Analysis enables intimate insight into bone strain of the femur during gate. The recordings of strain show four distinct points during a gate cycle: 1) Mid-stance, 2) Lift-off, 3) Mid-swing, 4) Touch-down. These points and their corresponding frames are shown in Figure 5c. Behavioral assays, gait analysis in particular, are useful tools to investigate pathogenesis and develop new therapeutics for musculoskeletal disorders such as osteoarthritis. Therefore, a basic requirement is that investigative devices do not alter gait performance of the subjects, which is not easily achieved with tethered approaches. Due to the low-profile and mechanical compliance, osseosurface electronic implants do not affect the subject’s gait, as revealed by deep neural network analysis of rats with implants (Figure 5d-e, details in the Methods section). The heat maps displayed in Figure 5d indicate that the trajectories of the paw of naïve and experimental subjects with implants remain qualitatively the same and the spatial distribution of paw velocity is unchanged at various stages of the study. Spatiotemporal gait characteristics, including stride frequency, stride length and duty factor, presented in Figure 5e, provide quantitative evidence that normal gait is sustained after implanting the osseosurface electronic device. The welfare of the test animals is also reflected by the steady weight-gain from two days post-surgery until the study is terminated (Supplementary Figure 21). In addition, histological analysis shows that the subcutaneous implantation of the device body in the lumbar
vertebra region, is surrounded with fibrous tissue containing blood vessels without any evidence of inflammation or significant foreign body response after two weeks of in-vivo operation (Supplementary Figure 22).

Figure C - 5. In-vivo studies of OSE in rats. a Photograph of the rear part of a rat featuring the µ-ILED attached on the femur illuminating through muscle and skin. b Temperature profile recorded in-vivo during the period of ~11.5 hours following the implantation surgery. Inset, schematic of the rat residing in the home cage. c Time synchronized strain recording and corresponding video frames of the rat gait cycle. d-e Comparison of the rat’s gaits at different stages of the study: before surgery, 1 week after surgery and 2 weeks after surgery: photograph of rats walking on the treadmill overlaid with the trajectory of the ankle. Color of the dots represents the velocity of the ankle (d). Key parameters – stride frequency (Before: mean = 2.4293, SD = 0.4057, n = 7; 1 Week: mean = 2.1843, SD = 0.1692, n = 7; 2 Week: mean = 2.3336, SD = 0.197, n = 7) stride length (Before: mean = 9.3812, SD = 1.749, n = 7; 1 Week: mean = 9.1175, SD = 2.2686, n = 7; 2 Week: mean = 8.8466, SD = 1.0577, n = 7), and duty factor (Before: mean = 0.6558, SD = 0.2689, n = 7; 1 Week: mean = 0.6189, SD = 0.118, n = 7; 2 Week: mean = 0.6182, SD = 0.0585, n = 7) – that characterizes the rat gait before and after implantation (e).
2.6 Permanently attached Osseosurface electronics

The ultrathin electronic platform enables direct lamination onto the bone which provides the opportunity to attach devices permanently to gather information on bone health long term. Because of cell turnover any glue will exhibit limited lifetime and continuously degrading biointerface quality. A solution to this problem is to directly grow osseosurface devices to the bone using of calcium phosphate ceramic (CPC) particles\textsuperscript{41}. For the use in animal subjects fast adhesion is critical to enable accelerated experimental timelines, which can be accomplished by the addition of transforming growth factor beta 1 (TGF-\(\beta\)1), an osteogenic protein that enables rapid bone bonding to the CPC particles\textsuperscript{42}. We use this scheme to bond CPC particles to the osseosurface electronics using implant grade epoxy, and subsequently applying TGF-\(\beta\)1 to the CPC particles. The resulting layered device composition is shown in Figure 6a. The exposed particles enable effective bone bonding after implantation and temporary fixation with resorbable sutures (Figure 6b). The efficacy of this approach can be observed in vivo by recording the average delta strain values of gait on a treadmill. Figure 6c shows the evolution of the delta strain over the course of several weeks, here we can observe weak bonding of the stain gauge to the bone for the day of the surgery with rapid increase in delta strain values in the following days and a stable attachment after a week (Supplementary Figure 23). The successful growth of the device to the bone can also be observed after explanation (day 27) and cross-section preparation and subsequent secondary electron imaging (Figure 6d and Supplementary Figure 24), details in methods section. The micrograph shows successful growth of the bone to the particles that permanently affix the flexible electronics enabling chronic recording of bone health. The chronic application of these devices enables a multitude of new studies for example strain mediated bone remodeling with experimental
paradigms that involve extensive mobility of subjects and longitudinal studies with multiple co-habitating subjects which is facilitated by complete recovery after implantation of the wireless and battery free device as shown in Figure 6e.

![Figure C-6](image)

**Figure C-6. Promotion of osteogenesis using surface-engineered calcium phosphate ceramic coatings.**

*a* Photograph of the bottom coated with CPC and a side view diagram of layer composition of the strain gauge sensor with CPC. 

*b* Image of device 3 weeks post implantation. 

*c* Wireless strain gauge data collected over 2 weeks post implantation showing stable CPC adhesion. 

*d* Single electron microscope image of cross section of CPC adhesion to bone. 

*e* Animal healing after implantation.

### 2.7 In-situ studies in large animal models.

The scalability of our platform enables the use in large animal models with minimal modifications. Immediately after euthanization, following the University of Arizona Institutional Animal Care and Use Committee approved protocol (16-202 IACUC), in-situ operation of devices designed for large animals are demonstrated on sheep humeri where small footprint and soft mechanics of the device enable conformal application to the bone surface (Figure 7a and Supplementary Figure 25a-b). The thermography function allows for continuous monitoring of local temperature as an important indicator of subject health throughout the surgical procedure. As shown in Figure 7b distinct features can be identified and correlated to events such as closing and
re-opening of the incision. Devices implanted deep (> 5 cm) in the body are warmed significantly faster to a saturation level close to the core body temperature (Supplementary Figure 25c-d) than those implanted shallower (~2 cm). The wireless strain recording capability through thick tissues is validated while the humerus is loaded in 3-point bending (Figure 7c), successfully capturing the bending events with well-discernable loading, and unloading phases despite considerably lower strain than those noted during gait measured from active sheep43.

3. Discussion

Wide dissemination of osseosurface electronics requires an implementation strategy that can be easily integrated into existing orthopedic surgical procedures. We demonstrate a device attachment strategy that is assisted by a 3D printed applicator and can be accomplished within ten minutes, minimally altering existing surgical protocols44,45 (Figure 7d, details in Methods section). The applicator can be customized to match localized anatomic structure and device dimensions (Supplementary Figure 16), minimizing the impacts on the surrounding tissues, and providing a path towards implementation as a diagnostic tool following routine surgical procedures.

Easy readout with industry standard RF protocols and rapid attachment with chronic interfaces enable osseosurface electronics to provide significant opportunities for the direct measurement of crucial indicators of bone health in real time. Consequently, this allows a point-of-care solution to monitoring post-fracture rehabilitation and managing musculoskeletal conditions. Here, we demonstrate the successful device operation using an NFC-enabled smartphone through tissues, as shown in Figure 7e where the sensor signals from a device covered by a piece of porcine skin (~1 cm thick) simulated human tissue on the tibia are directly visualized in real time as the smartphone is used to provide power to and wirelessly communicate with the
device (Supplementary Video 3). These results demonstrate the feasibility of operating osseousurface electronics with a smartphone in an at-home setting. Locations covered with several muscle layers such as the femur in larger animals, can be accessed with a dedicated reader solution with near-field-enabled clothing for efficient power transfer and continuous communication during everyday behaviours. Figure 7f plots the relative data rate and harvested power as functions of tissue thickness between the reader and the device, showing no degradation in either data rate or available power level at the devices at tissue thickness up to 11.5 cm.

This highlights successful wireless power transmission and data communication with a battery-free and soft implantable device through thick tissues, which enables implantation on almost any location on the human skeletal system.

The miniaturized form factors, soft mechanics, versatile sensing/stimulation options, as well as robust wireless power harvesting and communication capabilities make osseousurface electronics a powerful platform to establish direct and chronic bi-directional interface with the musculoskeletal system. This offers unprecedented opportunities for mechanistic studies of osteogenesis and pathogenesis of musculoskeletal diseases, as well as the development of new types of diagnostics and therapeutics. Furthermore we demonstrate the successful attachment of the Osseousurface electronic biointerface to the bone utilizing surface engineered CPC particles, an important aspect of this technology that enables recording on the order of many years enabling device operation for the lifetime of the subjects without requiring secondary surgery. This chronic platform can then enable acquisition of holistic data of bone health status and closed loop therapeutic intervention to facilitate treatment and rehabilitation.
4. Methods

4.1 Device fabrication.

Flexible circuitries were fabricated by UV (355 nm) laser ablation (ProtoLaser U4, LKPF, Germany) using a sheet of copper clad polyimide foil (Dupont, Pyralux AP8535R, copper/Polyimide/copper, 17.5 μm/75 μm/17.5 μm) as substrate. Subsequent sonication in solder flux and isopropyl alcohol removed the surface oxides formed during laser ablation. Surface-mount components, including passive components such as resistors (0201, 0.6 mm x 0.3 mm), capacitors, Schottky diodes (Skyworks Inc.), Zener diodes (Comchip Technology Corp., 5.6 V), µ-ILED (red, ES-AEHRAX10, EPISTAR) and NTC thermistor (NTCG064EF104FTBX, TDK), as well as active components such as MOSFET (PMZ130UNE, Nexperia), low-dropout linear regulators (LDO, TCR2DG18, Toshiba), instrumentation amplifier (INA, AD8235, Analog Devices) and NFC SoC (RF430FRL152H, Texas Instruments), were manually placed on the flexible circuit and reflowed with low temperature solder (Indium Corp.). A metal foil strain gauge (N2A-06-S5182N-10C/E4, Micro-Measurements) was then integrated by using a chisel tipped soldering iron. Finally, the device was baked at 120 °C in an oven for 30 minutes to remove residual flux and solvents used during the assembling process. Devices were treated with silane (A174, Sigma Aldrich) and encapsulated with two layers of 9 μm Parylene-C using a Parylene coating system (Parylene P6, Diener electronic GmbH). Devices were then coated with PDMS (Sylgard 184, Dow Corning) by dip coating.

4.2 Circuit simulation.

LTspice XVII was utilized to simulate the electrical characteristics of the strain sensing and temperature sensing analog front-ends. The circuit diagram and choice of components are
shown in Figure 5. The output voltage of the Wheatstone bridge and instrumentation amplifier were selected as the output parameters. To simulate the basic characteristics of the strain sensor circuit (Supplementary Figure 5a-b), resistance of the strain gauge was varied sinusoidally from 999 Ω to 1001 Ω with a frequency of 20 Hz. To simulate the strain sensing performance with various values of the bridge resistors (Rb) (Supplementary Figure 5c-d), Rb was varied linearly from 1 kΩ to 10 kΩ, at four values of the gauge resistance (998 Ω, 999 Ω, 1001 Ω and 1002 Ω). To simulate the basic characteristics of the temperature sensing circuit, the thermistor resistance was varied from 72.32 kΩ to 46.49 kΩ corresponding to the temperature range of 32 °C - 42 °C.

4.3 Device characterization.

Wireless power harvesting capability: RF power in the range of 2 - 8 W was provided by a long-range RFID reader module (Feig Electronic GmbH, ID ISC.LRM2500-A). The primary antenna was connected to a tuning/matching circuit board and tuned at 13.56 MHz with a voltage standing wave ratio (VSWR) below 1.5. A 1-turn circular primary antenna with diameter of 20 cm was used to power the devices for large animals, while the devices for rodents were powered by two types of primary antenna: a 45 cm x 12 cm, 2-turn (at heights of 3 cm and 6 cm) coil that encloses the rat treadmill cage, and a 26 cm x 33 cm, 2-turn (at heights of 3 cm and 9 cm) coil that encloses the rat home cage.
The completed wireless power harvesting module was tuned to 13.56 MHz using a spectrum analyzer with reflection bridge (SSA 3032X, Siglent). Capacitors were added to tune the antenna to reach peak attenuation at 13.56 MHz. The capacitors are used to calculate inductance of the antenna by matching the reactance of the capacitor and inductor at 13.56 MHz. Power and voltage characterization of the antenna was tested by placing the device in the center of the test arena in parallel with the arena floor. The rectified voltage was then recorded with a digital multimeter while the electrical load was varied from ~50 Ω to ~5 kΩ.

The spatial distribution of harvested power was measured at a fixed load (300 Ω or 900 Ω) while the device was placed at various locations and different heights (3 cm and 6 cm) in the test arena. The angular dependence of the power harvesting capability was measured by varying the
angle of the device with respect to the arena floor from 0° to 70° using a rotational jig. The
dependence of harvested power on curvature radius was measured by conforming the device to
curved surfaces of 3D printed objects with varying radii of curvature (1.6 cm - 3.2 cm).

Wireless data communication: Wireless data reading was accomplished by ISOStart
(V10.09.00, Feig Electronic GmbH). In order to mimic the in-vivo environment, the device was
placed at the bottom of a 250 ml beaker filled with 1x PBS (Sigma Aldrich) solution. For the large
animal device, data rate was measured while the handheld primary antenna was held at various
heights from the device. For the rodent device, data rate was measured at representative locations
in the test arenas (Supplementary Figure 7). Long-term data recording was performed using the
rodent device and the 45 cm x 12 cm primary antenna over a ~42-hour period while the device
was immersed in PBS solution and constantly moving back-and-forth (25 cm s⁻¹) on a custom-built
rat treadmill. A custom-built thermometer (LMT70, Texas Instruments) was used to monitor the
environment temperature. Device lifetime estimation was calculated using an activation energy of
57800 J mol⁻¹ based on device failure at 90 °C and latest measured operation of device at 60 °C.
Using the Arrhenius scaling equation, we estimated a device lifetime of 205 days at 37 °C⁴⁹.

Characterization of the wireless strain sensor: Bench-top tests of the wireless strain sensor
were performed using an explanted sheep’s femur. The periosteum of the mid-diaphysis was
removed from the femur, and the metal foil strain gauge (N2A-06-S5182N-10C/E4, Micro-
Measurements) of the wireless osseosurface system was attached to the femur using a
cyanoacrylate-based adhesive (M-Bond 200, Micro-Measurements). A wired strain gauge was
subsequently bonded on top of the wireless gauge following the same procedure using a
stereomicroscope to ensure overlap and alignment of the sensing elements. The sheep femur was
loaded in four-point bending configuration using a servo-hydraulic materials testing system (Series
810, MTS Systems Corporation) while recording load and strain from the wired sensors using standard data acquisition system (System 8000 and StrainSmart, Micro-Measurements). Measurements from the wireless gauge were recorded with a handheld antenna using the Feig reader and ISOSTart. Various load profiles were tested, including a linear ramp load, and cyclic loading with a sinusoidal wave pattern, a square wave pattern and a triangle wave. The femur was loaded to a peak load of 190 kg at rates ranging from 5 - 60 kg s\(^{-1}\) in each profile.

Characterization of the wireless optical stimulation module: The current-voltage (I-V) characteristics of the \(\mu\)-ILED was recorded with a source measurement unit (SMU, Keithley 2450) operating in the linear sweeping mode. The optical power was measured with an integration sphere (OceanOptics FOIS-1). The current consumption of the \(\mu\)-ILED was measured with an Oscilloscope (Siglent SDS 1202X-E) measuring the voltage across a 10 \(\Omega\) resistor in series with the \(\mu\)-ILED. The wireless circuit uses a MOSFET (PMZ130UNE, Nexperia) to drive the \(\mu\)-ILED with programmed pre-defined frequencies and duty cycles stored in the \(\mu\)C (ATTiny 13A, Microchip Technology). Total current consumption of the device was measured using a benchtop power supply (1.8 V) with an Oscilloscope (Siglent SDS 1202X-E) recording the voltage drop across a 10 \(\Omega\) resistor.

Characterization of the wireless thermography: The wireless temperature sensor was immersed in a water bath whose temperature was varied from 33 °C to 41 °C using a hotplate and monitored by a commercial thermocouple digital thermometer. The sensor signal was wirelessly recorded with a handheld antenna using the Feig reader and ISOSTart and used for sensor calibration.

In order to characterize the resolution of the wireless thermographic biointerface, the copper on the bottom side of the Pyralux substrate was laser ablated to form a micro-heater beneath
the NTC thermistor (design of the micro-heater is shown in Supplementary Figure 9a). The micro-heater was wirelessly powered by the osseosurface device and the on board voltage regulator was used to drive PWM controlled 1.8V with a heater element resistance of 5.36 Ω that was characterized prior with a SMU (SMU, Keithley 2450) enabling a defined heater output. The μC was used to control a programmed sequence of duty cycles varying from 4% to 12% to control the MOSFET that drives the micro-heater. The fully encapsulated circuit was immersed in a water bath and a handheld antenna was used to wirelessly power the device and retrieve temperature recordings from the NTC. The resolution of the temperature sensor was subsequently determined by the smallest sensor response that could be distinguished from the background noise.

A circuit with co-located NTC thermistor and μ-ILED (~0.1 mm apart) was fabricated and used to demonstrate the capability to directly measure the thermal impacts of optical stimulation. The thermistor and μ-ILED were immersed in a PBS bath to mimic the in-vivo environments and to prevent the device from overheating at high optical power. A function generator (Siglent, SDG 1032X) was used to drive power to the μ-ILED to test μ-ILED heating of surrounding tissue using a micro-temperature sensor collocated by the μ-LED varying voltage (1.85 V, 2 V and 2.12 V), frequency (5 - 40 Hz) and duty cycle (5 - 80%), while the temperature sensor signal was wirelessly read out with a handheld antenna.

Mechanical durability of serpentine interconnects: The serpentine interconnects were mounted on a custom-built stretching stage and subjected to ~250% strain and stretched cyclically for 10,000 cycles. The resistance of the serpentine copper traces was measured with a digital multimeter.

The strain gauge of an osseosurface electronic system was bonded onto a piece of Kapton foil (~75 μm thick). The Kapton foil was mounted on the stretching stage and subjected to cyclic
bending with a radius of curvature of ~2 cm while the serpentine interconnects were stretched to various lengths (ΔL ~3 cm and 5 cm) with a separate stage. The strain sensor signal was wirelessly recorded with a circular primary antenna (20 cm in diameter). The same measurement was repeated after the serpentine had been stretched for 5,000 cycles and 10,000 cycles.

4.4 Mechanical simulation

Ansys® 2019 R2 Static Structural was utilized for static-structural Finite Element Analysis (FEA) simulations to study the strains induced in the copper traces of the serpentine interconnects, and the effectiveness of mechanically isolating the strain gauge from other parts of the device. The components of the devices, including the copper and constantan traces, Pb-free solder, polyimide (PI), and Parylene-C encapsulation layers, were modeled using the layouts used in device design. The mechanical properties (Young’s Modulus (E) and Poisson’s Ratio (ν)) used for the simulation were: EPI = 4 GPa, VPI = 2.7579, ECu = 121 GPa, VCu = 0.34, EConstantan = 162 GPa, VConstantan = 0.32, EParylene = 2.7579 GPa, VParylene = 0.4, ESolder = 43 GPa, VSolder = 0.29.

Simulation of strain in serpentine interconnects: The model was simulated using the following meshing parameters: Program Controlled Nonlinear Mechanical Elements with an Element Size of 8.0 x 10^-5 m, a Body Sizing insert condition with Element Size 1.0 x 10^-5 m applied to all the traces, and a Body Sizing insert condition with Element Size 2.0 x 10^-5 m applied to the PI layer. The simulation was performed by fixing one end of the selected serpentine segment and applying a displacement of 5.75 mm upwards to the other end as shown in Supplementary Figure 15, producing a deformation replicating that observed in bench top testing of the serpentine interconnects (Figure 4b).
Mechanical isolation of the strain gauge: The model was simulated using the following meshing parameters: Program Controlled Mechanical Elements with an element resolution of 2, and a Body Sizing insert condition with Element Size 2.0 x 10^-5 m applied to all the traces. Two simulations were performed, both with 1000 µε applied to the bone model by fixing one end while displacing the other end by 7.9 x 10^-6 m, as shown in Supplementary Figure 15: i.) no strain was applied to the serpentine interconnects; ii.) the serpentine interconnects were subjected to 3D displacements (x: 1 mm, y: 3 mm, and z: 3 mm. Details in Supplementary Figure 10b).

4.5 Animal studies

All animal experiments were performed following a University of Arizona Institutional Animal Care and Use Committee (IACUC) approved protocol (Sheep: 16-202, Rat: 19-572). In-vivo study in rats: Five male 450-gram Sprague Dawley rats were used. The implanted devices were sterilized using ethylene oxide and aerated for 24 hours prior to placement. Rats were anesthetized using isoflurane and were given a subcutaneous injection of 1.0 mg kg^-1 Buprenorphine SR prior to surgery. A 2 cm incision was made along the midline of the back over the lumbar spine. A 1 cm incision was made over the lateral thigh, and the anterior surface of the femur was exposed subperiosteally through a lateral approach. The device was placed in a subcutaneous location through the back. Passage of the strain gauge into the thigh via a subcutaneous tunnel was facilitated by the serpentine interconnect. The strain gauge was fixed to the femur using a cyanoacrylate adhesive (M-Bond 200, Micro-Measurements). For implantation of CPC coated devices in small animals, a 5-0 vicryl suture lassoed around the biosensor was used to pass the sensor from the lumbar region through the subcutaneous tunnel to the thigh. This lasso technique was used to avoid manipulation of the device with sharp surgical instruments and
minimize the risk of damage to the encapsulating layers. CPC coated gauges were secured to bone using two 5-0 vicryl sutures without adhesive. Layered closure was performed using 5-0 vicryl for fascia and 4-0 Quill for subcutaneous tissues prior to recovery from anesthesia.

During recovery, strain and temperature measurements were continuously recorded with the 26 cm x 33 cm primary antenna while rat behavior was recorded via a webcam. After 2 days, measurements were collected while rats walked on a custom-built treadmill at 25 cm s\(^{-1}\). Sensor signals were recorded wirelessly with the 45 cm x 12 cm primary antenna while high speed (1920 x 1080, 120 frame per second, iPhone 6S) videos were recorded with a camera placed ~50 cm from the treadmill for the purpose of deep neural network of the gait. Before each exercise session, the rat received a minimum of 20 minutes of habituation to the treadmill. Strain changes were observed during loading and unloading of the femur (Figure 5c). After 2 weeks the rats were euthanized. Following euthanasia device placement was characterized by scanning rats at 20 µm resolution using a Siemens Inveon micro-CT Scanner. For histology, tissue surrounding the implanted device was excised and imaged using an optical microscope (Wild M3Z Stereozoom Microscope, Leica) with an attached camera (iPhone 12 Pro, Apple) (Supplementary Figure 22a-c), fixed in 10% formaldehyde for 24 hours and embedded in paraffin. Ten-micron sections were cut and stained with hematoxylin and eosin (Supplementary Figure 22d-f). Slides were viewed using a Nikon microscope coupled to a Nikon DS-Fi1 camera at magnifications ranging from 20x - 100x.

In-Situ study in sheep: One male 2.5-yr-old sheep was used to confirm function of the device in a large animal. The sheep was a control used in another study and had a 4.2 cm femoral defect created 6 months prior to use. Device placement occurred following administration of euthanasia medications for the other study and was completed prior to significant changes in body
temperature. The anterior surface of the mid-diaphyseal humerus was exposed subperiosteally through a lateral approach. The wireless device was fixed to the anterior surface of the humerus deep to brachialis. The facia and skin were closed with running sutures. Measurements of strain and temperature were collected continuously using a handheld antenna placed on the skin for 20 minutes following surgical placement of the device. Function of the strain sensor was confirmed by loading the sheep’s humerus in three-point bending (Figure 6c).

4.6 Deep neural network analysis

DeepLabCut (version 2.2.b6) was used to perform deep neural network analysis. The neural net was trained with a 1-minute video clip where 200 frames were extracted as training material. The training session was performed with 200,000 iterations on the High-Performance Computer of University of Arizona. After training, a 3-second video clip with consistent gait was tracked and analyzed by the software to extract the coordinates of the left hind paw for each frame. The timestamp and coordinates were subsequently utilized to calculate the spatiotemporal gait characteristics, including stride length, stride frequency, duty factor, and the velocity of the paw.

4.7 Osseosurface electronics bone attachment

An applicator comprised of a 3D-printed frame and an elastomeric membrane (Supplementary Figure 26) was utilized to facilitate fast attachment of the device on the bone surface. Before attaching the device, the surface of the bone was abraded. The device was first attached to the applicator with the top side in contact with the elastomeric membrane. Adhesive agent (M-Bond 200, Micro-Measurements) was then applied to the bottom side of the device. The applicator was designed to make surgical process in large animals easier by improving ability to
conformally mount the strain gauge to the bone in one step. Once placed, the device was then
firmed pressed against the bone with an even force until the adhesive agent cured. Finally, the
applicator was carefully peeled off with minimal out-of-plane sheer force between the membrane,
device encapsulation, and adhesive agent.

4.8 Calcium phosphate ceramic coating

Implant-grade epoxy (Master Bond EP42HT, Master Bond, Inc., Hackensack, NJ) was
prepared according to the manufacturer's instructions and a thin even layer was blade coated to the
bottom surface of the strain gauge (N2A-06-S5182N-10C/E4, Micro-Measurements). Spherical
crystalline calcium phosphate ceramic (CPC2, CeraMed) was added to cover the surface of the
epoxy and silicone rubber was used to gently apply downwards pressure on the CPC particles
while curing the epoxy for 24 hours at room temperature. All CPC coatings were inspected for
surface exposure without epoxy coating to enable bond to the bone. Devices were coated with
TGF-β1 to accelerate bone to CPC bonding.

4.9 Scanning electron microscope imaging of CPC – bone adhesion

A rat after 27 days post-implantation was euthanized, and the femur was explanted along
with the attached strain gauge. The bone was cleaned, removing any soft tissue and fixed using a
neutral buffered formalin for 2 hours. The bone was dehydrated in a solution of ethanol (EtOH)
starting at 70% for 2 hours and increasing concentrations for at least 2 hours as follows: 80%
EtOH, 95% EtOH, 95% EtOH, 100% EtOH, 100% EtOH, xylenes, 100% EtOH. The bone was
embedded in methyl methacrylate (Technovit 9100 kit, Kulzer) through a pre-infiltration process
for 2 hours at room temperature and an infiltration process within a vacuum chamber for 15
minutes to remove any bubbles. The embedded bone is then cured at -4C over night. The bone and strain gauge were cut exposing the cross-sectional view. The cross-section surface was smoothed and polished using a grinding wheel (GP-25, Leco) from 120 grit to 600 grit. The surface was gold sputtered (Hummer 6.3, Anatech USA) allowing for a gold thickness of 9 nm. SEM images were taken with various magnifications (60x, 120x, 280x, 300x, 600x) around the bone strain gauge interface using (Inspect S50, FEI) operating at 30kV.

4.10 Device operation in deep tissue

Slits of ~3 cm length were cut into the porcine tissues and layers of tissue were stacked to increase device – primary antenna distance, as shown in Supplementary Figure 27 for a test at 8 cm device- primary antenna distance. The rectified voltage and data rate were recorded with a handheld primary antenna while the device was loaded with 1 kΩ and inserted into the slits to result in device- primary antenna distances from 0 cm to 11.5 cm.

Real-time data visualization through tissues: A device was attached on a sheep’s humerus following the device attachment procedures described above, and subsequently covered by a piece of porcine skin (~1 cm thick). An NFC-enabled smartphone running a custom software was brought in proximity to the porcine skin. The NFC connection was subsequently established, and the sensor signals were visualized on the smartphone in real time (Supplementary Video 3). To demonstrate real time readout, a device was attached onto a model of a human femur, then covered with a piece of porcine skin, and subsequently operated through the tissue with a smartphone while the femur model was strained to induce signal change (Supplementary Video 4).
5. References


