Monitoring regional tissue oxygenation in animal models and potentially in human subjects can yield insights into the underlying mechanisms of local O2-mediated physiological processes and provide diagnostic and therapeutic guidance for relevant disease states. Existing technologies for tissue oxygenation assessment involve some combination of disadvantages in requirements for physical tethers, anesthetics, and special apparatus, often with confounding effects on the natural behaviors of test subjects. This work introduces an entirely wireless and fully implantable platform incorporating (i) microscale optoelectronics for continuous sensing of local hemoglobin dynamics and (ii) advanced designs in continuous, wireless power delivery and data output for tether-free operation. These features support in vivo, highly localized tissue oximetry at sites of interest, including deep brain regions of mice, on unthethered, awake animal models. The results create many opportunities for studying various O2-mediated processes in naturally behaving subjects, with implications in biomedical research and clinical practice.

INTRODUCTION

Regional tissue oxygenation reflects the balance between O2 supply and demand and represents a ubiquitous hallmark in various physiological and pathological processes (1). Of particular interest are highly localized tissue oxygenation levels due to relevance in the interplay between O2 dynamics and neural activity, tissue perfusion, tumor microenvironment, wound healing cascades, and many others, as shown by studies on small animal models (e.g., mice or rats) (2–5). Systems for reliable monitoring could lead not only to an improved understanding for O2-mediated biological processes but also to important insights in clinical diagnostics and therapeutic guidance. Existing methods for the direct (in the form of O2 partial pressure) or indirect (in the form of changes in the concentration of oxygenated hemoglobin, [HbO2], and deoxygenated hemoglobin, [Hb]) assessments of localized tissue oxygenation in animal models have some combination of limitations associated with inability to operate at substantial depths beneath the body surface [near-infrared spectroscopy (NIRS) or cerebral oximeters], requirements for physical tethers (O2 electrodes, optical fibers, or bulky head stages), and/or need for anesthetics or special apparatus [brain oxygenation level–dependent magnetic resonant imaging (BOLD-MRI) and electron paramagnetic resonant spectroscopy (EPR)] (6, 7). These disadvantages can lead to confounding effects associated with altered oxygenation levels due to anesthesia (8) and/or with physical constraints (9, 10) on the natural behaviors of animal models, and associated inability to perform studies during social interactions.

Here, we present a miniaturized, fully implantable, wireless oximetry system that consists of a filamentary measurement probe (cross-sectional area less than 400 μm × 200 μm) interfaced to a small electronic module (lateral dimensions of less than 1 cm2 and thickness of 1 mm), with unique capabilities that overcome these challenges. The sensing filament includes high-performance optoelectronic components [microscale inorganic light-emitting diodes (μ-ILEDs) and a microscale inorganic photodetector (μ-IPD)]. The electronic module supports wireless power harvesting, circuit control, and data communication to external receivers. The sensing exploits well-known differences in the optical properties of HbO2 and Hb to quantify local changes in their concentrations (Δ[HbO2] and Δ[Hb]) as a means to estimate regional tissue oxygen saturation levels [rStO2 ≈ 100% × [HbO2]/([HbO2] + [Hb])] in small tissue volumes defined by the illumination profiles at the tip end of the sensing filament. Wireless power harvesting via magnetic resonant coupling and data transmission by infrared (IR) communication use small-scale electronic designs as an important extension of recently developed implantable platforms for optogenetic modulation (11–17) and photometric measurements (18). The miniaturized form factors (with the injectable parts similar in sizes to those of other minimally invasive techniques), the lightweight construction (~80 mg), the mechanically compliant designs, and the bio-compatible encapsulation materials facilitate implantation, minimize tissue damage, and provide potential capabilities in robust, chronic operation. These features support unique capabilities in continuous localized rStO2 measurements from devices subdermally implanted in...
peripheral tissues and even in targeted regions of the deep brain in both anesthetized and awake, freely moving animal models, with the ability to monitor transient changes in oxygenation. Resultant capabilities open up possibilities for studying a broad range of O2-mediated, location-sensitive processes on naturally behaving subjects for both biomedical research and clinical practice.

RESULTS
Design features
Figure 1A shows an exploded schematic illustration of a fully implantable oximeter of the type described above. The platform incorporates two functional modules: (i) an injectable filament for real-time rStO2 sensing (in the golden dashed box); (ii) a thin, battery-free module that supports wireless power delivery and control, photodiode amplification and analog front end, and optical data communication (in the green dashed box). The sensing filament exploits optoelectronic designs typical of reflectance-mode, rStO2 oximeters [e.g., NIRS (19, 20)], with a pair of µ-ILEDs that emits, in a time multiplexed fashion, at complementary wavelengths tailored toward efficient measurement of [HbO2] and [Hb] levels. A single photodiode measures the attenuated light backscattered by hemoglobin molecules in blood associated with the surrounding tissues and vasculature. The fabrication procedures represent extensions of those recently reported for wireless systems for optogenetic modulation (11–17) and photometric measurements (18). In brief, the techniques of microtransfer printing with elastomeric stamps [made from polydimethylsiloxane (PDMS)] enable precise assembly of two µ-ILEDs (with dimensions of 270 μm × 220 μm × 50 μm and 240 μm × 240 μm × 100 μm) and one µ-IPD (with dimensions of 100 μm × 100 μm × 5 μm) onto a substrate of polyimide (PI; thickness, 75 μm). Photolithographically defined traces of gold/copper (Au/Cu; width, 20 μm; thickness, 700 nm) form interconnects between these optoelectronic components. Depending on application requirements, the two µ-ILEDs can be located on opposite or the same side as the µ-IPD, as shown in Fig. 1 (B and C). The former configuration exploits a dual-layered design with µ-ILEDs and µ-IPD separated by a 7-μm-thick, insulating layer of a photo definable epoxy (SU-8; Fig. 1B). The supporting filament has a width of ~380 μm and a thickness of ~80 μm (~200 μm for the entire filamentary probe), with a length to match the application. These probes are comparable in cross-sectional area to those in conventional, tethered techniques for tissue O2 measurements, such as fiber oximetry (e.g., diameters of ~250 μm) and polarography (e.g., diameters of 200 to 300 μm for O2 electrodes) (6), and with traditional fibers used for optogenetics (outer diameters of 230 to 480 μm) (21, 22). The low stiffness of the filaments [between two and three orders of magnitude smaller than those of typical optical fibers (23, 24)] allows compliant mechanical interfaces with soft tissues, thereby reducing disruption due to implantation and chronic use. These collective features enable deployment of these systems for rStO2 sensing at sites within sensitive tissues, including regions of the deep brain, in a wide range of animal models, including mice. The latter configuration includes two µ-ILEDs on the same side as the µ-IPD (Fig. 1C) and is most amenable to use outside the brain, especially in locations that require highly localized sensing of rStO2.

In both cases, bioinert coatings (a conformal coating of parylene with a thickness of 14 μm and, in some cases, an additional coating of PDMS with a thickness of ~10 μm) encapsulate the devices as barriers to biofluids to ensure their stable operation as chronic implants. The efficacy of these coating materials has been evaluated on related functional implants in the brains of living animal models in previous reports (14, 18, 25). Detailed descriptions of the fabrication procedures appear in fig. S1 and in Materials and Methods. Figure 1 (D and E) shows photographs and scanning electron microscopy (SEM) images of representative filaments, highlighting their small dimensions, particularly those with the dual-layered design [Fig. 1, D (right) and E (top)]. For use cases that require the sensing unit to be separated from the electronic module by a relatively large distance, the filament can be formed into a long, serpentine-shaped geometry (length of ~4 cm for this example, but selectable over a wide range) (26) to maintain system-level functionality with a high degree of mechanical flexibility and stretchability [Fig. 1, E (bottom) and F]. Additional illustrations and images appear in figs. S2 and S3.

Integration of the injectable filament with the electronic module occurs through low-temperature reflow soldering to yield a functional system (Fig. 1G). The electronic module incorporates wireless power harvesting via magnetic resonant coupling to an external antenna and wireless data communication by IR broadcast to a collection of photo-receivers (Fig. 1H). The harvesting unit includes (i) a loop antenna optimized for an operation frequency of 13.56 MHz, with minimum sizes defined by areas of ~0.9 cm2, with side lengths of ~1 cm, consisting of five turns of copper traces (widths and spaces of 70 μm), and (ii) a subsequent half bridge rectifier with a ceramic capacitor (4.7 μF) for waveform smoothing followed by a supercapacitor (2.2 mF) for buffering, as the basis for stable power supply at a voltage of 3 V through a low-dropout regulator (Fig. 1H, green dashed box). During operation, a low-power microcontroller (µC) defines the timing of activation/deactivation of the µ-ILEDs (in an alternating time-sequence manner), the sampling of the transmitted signals via the integrated analog-to-digital converter (ADC), and the timing of the IR LED for wireless communication (carrier frequency, ~38 kHz).

A miniaturized analog front end conditions and amplifies the response of the µ-IPD to attenuated, backscattered light from µ-ILEDs. External integrated data receivers (illustrated in the blue dashed box in Fig. 1H) that incorporate automatic gain control, band-pass filtering, and demodulation yield digital signal data streams from IR light transmitted from the electronic module. The high IR transparency of biological tissues allows effective operation even with the electronic module fully implanted subdermally. An external µC analyzes and time-stamps these digital signals and sends them through a serial communication link to a personal computer for data storage and analysis. In addition to wireless power harvesting, the wireless electronic modules can also be designed to incorporate small, lightweight, polymer lithium ion batteries (fig. S4) (18). These lightweight (as small as <0.1 g; fig. S5), subdermal implants offer capabilities for probing localized rStO2 at sites of interest in untethered animal models that lie outside of those possible with conventional technologies.

Optical and electrical characterizations
The estimation of rStO2 relies on the distinct absorption spectra of hemoglobin in the visible and NIR spectral range, depending on their oxygenation forms (HbO2 or Hb). In general, the ratio of HbO2 in hemoglobin molecules tends to increase at elevated O2 concentration but decreases under hypoxia. Figure 2A shows the molar extinction coefficient (ε) spectra of HbO2 and Hb according to reported data (27). Substantial changes in the spectral dependence of ε occur during the transition between HbO2 and Hb. The absorption spectra of hemoglobin solutions appear approximately as linear combinations of
Fig. 1. Miniaturized, fully implantable, wireless oximeters for rStO2 measurements. (A) An expanded view of the device platform including the electronic module (green dashed box; only parts of the electronic components are shown) and the injectable module (golden dashed box). (B and C) Schematic illustrations highlighting two representative filamentary designs: (B) dual-layered design for deep brain rStO2 sensing of mice and (C) single-layered design for highly localized rStO2 sensing in other tissue regions. (D) Left: Photograph of the dual- and single-layered filaments near a U.S. dime. Right: Optical and SEM images of the tip end of the dual-layered design (red dashed box in the left panel) with two μ-ILEDs placed as the opposite sides of the μ-IPD. (E) Photographs of (top) the dual-layered and (bottom) stretchable filamentary sensing modules at a tilted view. (F) Images of flexible and stretchable filaments with serpentine interconnects. (G) Integrated wireless, battery-free oximeters in operation mode with illuminating μ-ILEDs. (H) Block diagram of the electrical working principles. LDO, low-dropout regulator; AGC, automatic gain control; Supercap, supercapacitor. (Photo credit: Hao Zhang and Philipp Gutruf, Northwestern University)
those of HbO₂ and Hb as a function of rStO₂. The result correlates the rStO₂ (as a function of [HbO₂] and [Hb]) to measurable optical properties (i.e., light attenuation by hemoglobin under transmission or backscattering mode). Commercial rStO₂ oximeters that operate on the skin generally use LEDs with two or more wavelengths: (i) in the NIR regime (~700 to 850 nm) with relatively large penetration depths through skin related to the low ε of water and main chromophores in the skin, and (ii) below and above the isosbestic point (~800 nm) where the sensitivity to oxygenation of hemoglobin is high, due to considerable differences between ε(HbO₂) and ε(Hb) (20, 28). By comparison, the geometry of the oximeter platform introduced here involves a short source-detector distance, thereby allowing the use of red- and green-emitting µ-ILEDs (625 nm with full width at half maximum of ~10 nm and 540 nm with full width at half maximum of ~30 nm, respectively; Fig. 2A) that are more well aligned to commercially available, small-scale components. The red µ-ILED allows probing the spectral range where the difference between ε(HbO₂) and ε(Hb) is large [ε(Hb)/ε(HbO₂)] up to 10 in the range of 600 to 700 nm; fig. S6] to enhance the ability to measure Δ[HbO₂] and Δ[Hb]. The green µ-ILED, by contrast, probes a portion of spectrum where ε(HbO₂) and ε(Hb) are similar, thereby permitting evaluation of oscillations in the total hemoglobin concentration and elimination of the influence of an unknown background (20), which are insensitive to rStO₂ changes. Specifically, algorithms based on the modified Lambert-Beer law for diffusive media allow quantitative calculation of Δ[HbO₂] and Δ[Hb] and estimation of rStO₂ with priori approximations about the baseline values (Materials and Methods). Detection of attenuated backscattered light from both µ-ILEDs relies on a single µ-IPD with high sensitivity to visible light [external quantum efficiency (EQE), ~74% at 540 nm and ~82% at 625 nm; shown in Fig. 2B].

The fabrication of these custom µ-IPDs relies on GaAs-based epitaxial structures grown with precise control over the doping profiles (figs. S7 and S8) and follows from a series of steps in photolithography, etching, and microtransfer printing (18). Characteristics of the µ-ILEDs...
and the μ-IPD, such as the current-voltage curves, appear in fig. S9. The distance between the μ-ILEDs and the μ-IPD, also known as the interoptode distance, sets the characteristic depth associated with backscattered light that arrives at the μ-IPD and strongly affects the signal-to-noise ratio. In general, increasing interoptode distance extends the optical path and enlarges the probing volume, which enhances the variations in signals due to changes in rStO2. Increases in interoptode distance also, however, decrease the light detected by the PD due to strong absorption and scattering events that occur in turbid media (29), which ultimately increase the noise in the detected signals. The interplay between probing depth, sensing volume, and signal-to-noise ratio represents a challenge for conventional rStO2 oximeters. By contrast, the injectable platforms presented here allow sensing in deep tissue regions at a small interoptode distance (700 μm) with adequate signal-to-noise ratio.

Monte Carlo simulations provide quantitative insights into the photon distributions around the μ-ILEDs and into aspects of light detection by the μ-IPD at this interoptode distance. The models use optical properties characteristic of those of mouse brain tissue. Details on these simulations appear in Materials and Methods. Figure 2C shows the normalized emission intensity profiles of green and red μ-ILEDs as a function of distance. The penetration depth, or the location where the optical intensity decreases to e−2 or ~10% of the initial value, is around 0.4 and 0.5 mm for the green and red μ-ILEDs, respectively. The characteristic sizes of the illumination volumes are ~0.5 to 2 mm3 depending on the threshold light intensities (e.g., intensities at 10% or 1% of the initial values). The large absorption and scattering coefficients associated with brain tissue, the divergent illumination patterns of the μ-ILEDs, and the height differences between the μ-ILEDs and the μ-IPD lead to detected signals that are dominated by backscattered light, with little contribution (over five orders of magnitude lower compared to that from backscattered light, according to simulated data) from light that passes directly from the μ-ILEDs to the μ-IPD. On the basis of the measured EQE of the μ-IPD, the simulated photocurrents as a function of rStO2 across the physiologically relevant range (fig. S10) correlate qualitatively with data from in vivo experiments, as shown in the following section.

Efficient wireless power harvesting and reliable data transfer are critical features of continuous monitoring of rStO2 using the platforms described here. Resonant power transfer and stabilization schemes for chronic and robust operation of devices in optogenetics serve as inspiration for the approaches used here (25). Figure 2D shows the unregulated power output of the rectifier with increasing load in the center of an experimental arena with the dimensions of a typical mouse home cage and circumscribed with a dual-loop primary antenna (L × W × H = 25 cm × 15 cm × 10 cm; scheme shown in fig. S11) at a height of 3 cm with a radiofrequency (RF) power input of 4 W. The optimal working voltage for this antenna/rectifier combination is around 4 V, yielding a power of around 12 mW, which is sufficient for device operation (peak power requirement of 9 to 10 mW buffered by the supercapacitor and average power requirement of ~2 mW). Spatially resolved measurements with a shunt resistance of 3.3 kilohms (comparable to the system load) show harvesting capabilities that exceed 30 mW at the corners of the cage and reach minimum values of around 12 mW at the center (Fig. 2E). The available power at any location within the cage exceeds that needed for stable voltage output of 3 V.

The average power required for robust device operation is further reduced by power management schemes shown in Fig. 2 (F and G), which illustrates the time-resolved current consumption of the system. These schemes feature (i) “sleep” phases (~80% of the operational time) where most of the μC peripherals are off to minimize current consumption levels (below a few tens of microamperes) and (ii) the sampling and data transmission phases (~20% of the operational time) where the power requirements are 9 to 10 mW. Using this operational duty cycle, the average power consumption drops to around 2 mW, which leaves ample margin for power supply even across large (L × W × H = 30 cm × 30 cm × 20 cm) experimental enclosures. Here, brief bursts of data broadcast via IR at rates of over 27 Hz with 12-bit resolution. These rates far exceed those necessary to capture temporal variations in rStO2 associated with tissue perfusion and global O2 levels in animal models (well below 1 Hz) (30). As in recent work on wireless photometers (18), the IR data transmission scheme is stable even in non–line-of-sight scenarios when the millimeter-scale IR LED faces all cardinal directions in a mouse home cage equipped with external receivers at the corners. The use of IR, as opposed to a wavelength in the visible range, leads to minimal attenuation by tissues of animal models such as rodents. For instance, 950-nm IR light preserves ~47% of original intensity after passing through a piece of scalp from a sacrificed mouse (Fig. 2H and fig. S12).

**Tests of wireless oximeters with artificial blood solutions**

Basic evaluations of function of these wireless, battery-free devices use artificial blood solutions that contain different hemoglobin derivatives, as controlled simulations of in vivo assessments of rStO2. Commercial bovine hemoglobin powders contain predominantly methemoglobin (metHb) with Fe(III) centers and readily form aqueous solutions in phosphate-buffered saline (PBS) (e.g., 25 g liter−1, referred to as sol. 1 in Fig. 3A). This concentration is comparable to that of total hemoglobin in the peripheral blood and brain vascular systems of mice and rats (31, 32). Adding excess reducing agents (Na2S2O4, 8:1 in mass ratio to hemoglobin powders) to sol. 1 yields a solution with markedly increased absorbance at ~540 nm and reduced absorbance at ~625 nm (sol. 2 in Fig. 3A). The distinct optical absorption features of sol. 1 and sol. 2 result in substantial changes in detected photoresponses (as ADC values) associated with green (decreased by ~50% when switched from sol. 1 to sol. 2) and red (increased by ~40%) wavelengths, as measured by oximeters immersed in these solutions in plastic centrifuge tubes. Note that the output data are stable over time within ~1.5% (fig. S13) when wirelessly powered by magnetic resonant coupling. These changes are negligible in amplitude compared to those associated with expected variations in hemoglobin compositions.

Figure 3B shows the computed ratios of output data ([ADC (green)/ADC(red)]) of five solutions with different compositions (absorption spectra shown in fig. S14), where sol. 1 and sol. 2 represent discrete oxygenation states of hemoglobin. These values correlate well with variations in optical absorbance of these solutions. Measurements of sol. 1 and sol. 2 at various locations (including center and corners) in an experimental arena of 25 cm × 15 cm × 10 cm reveal the system-level performance, including power harvesting, oxygenation measurements, analog front-end processing, and data communication, under conditions relevant to the context of rStO2 measurements in freely moving animal models. The spatially resolved graph in Fig. 3C shows excellent stability of the ratios of output data associated with green and red wavelengths at different locations within the experimental arena (±2% to ±7% deviations, as represented by the error bars in Fig. 3B).
Fig. 3. Tests with artificial blood solutions and in vivo \( rStO_2 \) measurements on rodents. (A) Absorption spectra of two artificial blood solutions with different compositions. a.u., arbitrary units. (B) Correlation of the output photoresponse signals (red open circles; as the ratios of ADC values) from the wireless, battery-free oximeters, with the differences in optical absorbance [black solid squares; as the ratios of absorbance (Abs) at 540 and 625 nm] of five artificial blood solution samples. (C) Spatially resolved output signals of wireless oximeters measured from sol. 1 and sol. 2 at different locations in an experimental arena with the dimensions (25 cm × 15 cm × 10 cm) of a mouse home cage. (D) Scheme of an anesthetized rat highlighting the femoral artery and vein region (red and blue blood vessels, respectively). (E) Photograph of a wireless oximetry implant (battery-powered, with the injectable module outlined by white dashed lines) in the tissue region near the femoral artery of an anesthetized rat. (F) Estimated \( rStO_2 \) (red traces) in the tissue region [shown in (E)] of an anesthetized rat exposed to \( FiO_2 \) changes (black traces) between 100% (red blocks) and 8% (purple blocks). (G) Scheme of surgical steps of the subdermal implantation of wireless oximeters in mouse brain (yellow sections). Left to right: Insertion of the filament into the brain with opened scalp (circled by blue dashed lines) via a drilled hole; bending the electronic module followed by fixing it on skull; and closing the scalp with biodegradable sutures. (H) Photograph of a freely moving mouse with subdermally implanted oximeter in the brain. (I) Schematic illustration of the setup for deep brain \( rStO_2 \) measurements of a freely moving mouse. (J) Estimated \( rStO_2 \) changes (red traces) in the deep brain region of freely moving mice in a hypoxia chamber with precisely controlled \( FiO_2 \) profiles (black traces; oscillating between 8 and 21%) using battery-powered oximeters. Changes in the color of blocks (red, yellow, and purple) indicate the time for \( FiO_2 \) changes. (Photo credit: Philipp Gutruf, Northwestern University)
**In vivo rStO2 measurements on anesthetized and freely moving rodents**

These in vitro results establish the basis of in vivo assessments of localized rStO2 with probes implanted at sites of interest in living animal models, without the physical constraints of tethered hardware required by other systems. Encapsulated battery-powered devices implanted near the femoral artery (as indicated in the scheme shown in Fig. 3D) of anesthetized rats operate effectively (Fig. 3E). With related types of devices (14, 18, 25), the parylene/PDMS encapsulation scheme can support stable operation as implants in mouse models for 1.5 years. These results suggest potential capabilities in chronic operation of devices reported here. As the anesthetized rats (n = 4 animals; total number of measurements = 10) experience oxygenation challenges via a nose cone (i.e., inspired fraction of O2, FiO2, varied between hyperoxia (100% O2 with 2% isoflurane), hypoxia (8% O2), and normoxia (ambient air)), Δ[HbO2] and Δ[Hb] yield substantial changes in the measured data (fig. S15). For instance, decreased FiO2 leads to reduced photoresponses associated with the red emission because Δ[HbO2] < 0 and Δ[Hb] > 0. In comparison, the photoresponses from green light are less sensitive to oxygenation challenges (and thus rStO2), consistent with the optical absorption features (Fig. 2A and fig. S6) and the simulated results (fig. S10).

These extracted photoresponses (in the form of ADC values) allow the quantitative calculation of Δ[HbO2] and Δ[Hb] using algorithms and data processing strategies described in Materials and Methods, derived from a modified Lambert-Beer law for diffusive media. In brief, the methods include extracting high and low ADC values corresponding to the two operating wavelengths, low-pass filtering with a zero-lag digital Butterworth filter, and linear detrending to account for drifts of the raw data (e.g., fig. S15B). Compared to arterial blood oxygen saturation (SaO2; another vital sign related to oximetry used in clinic practices), a critical challenge in the estimation of rStO2 is the lack of a reliable “gold standard,” as rStO2 represents a weighted average of the oxygen saturation throughout all intravascular blood within the illuminated volume (33). Consequently, commercial rStO2 oximeters are typically used to monitor trends with pooled root mean square errors of ~±8% and relatively large variations among human subjects (19, 33). Despite these complications, the algorithm used here yields estimates of rStO2 (Fig. 3F) with assumptions for the total concentration of hemoglobin (~150 g liter\(^{-1}\)) and the baseline value of rStO2 (~60%) (31, 32). The temporal changes in estimated rStO2 coincide with the hyperoxia-hypoxia FiO2 cycles, with fast temporal response. An interesting observation is that the data suggest that the vascular system in this tissue region is more resistant to the transition from hyperoxia to hypoxia than the other way around (Fig. 3F and fig. S15). Similar “asymmetric” changes appear in photoacoustic imaging of rStO2 in the brains of anesthetized mice (34). Placing the same device at different distances (0.5 to 1 mm versus 2 to 4 mm) from the femoral artery of the same rat yields notable changes in the computed ratios of the output photoresponses (fig. S15C), likely due to spatial variations in rStO2 (35).

Advanced demonstrations involve real-time cerebral oximetry in the striatum of untethered, freely moving mice, as an example of a capability that would be difficult or impossible to replicate with existing direct or indirect O2 measurement technologies. Subdermal implantation of miniaturized, wireless, battery-free oximeters (Fig. 1G) in the mouse brain follows stereotactic surgical procedures described in previous reports (14, 18, 25). Figure 3G illustrates some of these steps, beginning with lowering the filament into a hole drilled at desired coordinates of the brain through the exposed skull, followed by fixing the probe with dental cement or cyanoacrylate to minimize relative movements in the brain (Fig. 3G, left), bending the electronic module and fixing it onto the skull (Fig. 3G, middle), and finally closing the scalp with bioresorbable sutures (Fig. 3G, right). Details appear in fig. S16. These fully implantable embodiments show reliable operation and continuous data recording capabilities throughout a mouse home cage circumflexed with a dual-loop primary antenna, thereby allowing measurements of rStO2 in deep brain regions of awake, freely moving mice (fig. S17). The implants introduce minimal injury to the brain during the surgery, and they prevent postoperative hindrances in the natural movements (Fig. 3H), as also evidenced in previous reports deploying related devices in optogenetic studies of mouse brain (14, 25). Mice implanted with battery-powered devices also show few changes in locomotor behaviors or social interactions (fig. S18), consistent with observations in adult mice using devices with similar weights (~0.5 g) and dimensions (18). Placing freely moving mice implanted with battery-powered devices (n = 3; total number of measurements = 9) in a hypoxia chamber (illustrated in Fig. 3I) with precise control over FiO2 (from 8% to about 21%) enables the continuous monitoring of cerebral rStO2 in deep brain regions (striatum). Figure 3J shows the calculated rStO2 during the FiO2 challenges of 21%-8%-21%-8%-21%-8%-21% and 21%-15%-8%-15%-21%-15%-8%-15%, respectively. The total concentration of hemoglobin and the baseline rStO2 at normoxia are assumed to be 0.1 mM liter\(^{-1}\) and 60% based on reported values (32, 36). The rStO2 levels do not completely recover to the baseline values (i.e., 60%) in 2 to 3 min after restoring FiO2 in the hypoxia chamber from 8 to 21%, possibly due to vasocostriction of microvessels of mouse brain in response to severe hypoxia (35, 37).

As with other invasive O2 measurement technologies, implantable oximeters can cause tissue damage during and after the implantation, especially in delicate regions of the anatomy such as the brain. Immuno-histochemical analyses of slices of mouse brains collected 4 weeks after implantation (at the location of 2 mm lateral and 4 mm deep to bregma; Fig. 4, A and B) reveal the effects. The small size, the compliant mechanics, and the biocompatible encapsulation of the filamentary sensors minimize tissue displacements and show normal immunoglogal response, as demonstrated in Fig. 4C. The estimated lesion sizes (360 μm × 240 μm) match the dimensions of the probes and are comparable to those of implants used in other O2 measurement techniques (e.g., diameter of ~250 μm for fiber oximetry and 200 to 300 μm for O2 electrochemistry). The battery-free implants reported here are also compatible with conventional imaging technologies such as micro-x-ray computed tomography (microCT). Despite certain device-induced artifacts, rendered images from slices in different orientations provide important insights on the location of the implants and the state of surrounding bones postoperatively, as shown in Fig. 4D. The sagittal (Fig. 4D, left) and coronal (Fig. 4D, right) views suggest that the electronic module laminates well on the skull where the small hole in the right panel corresponds to the location of the implantable filament. The relatively small extent of damage leads to minimal interruptions on the natural activities of mice, as suggested by the postoperative weight changes in a long term up to about 250 days (fig. S19).

**DISCUSSION**

The ultraminiaturized, lightweight optoelectronic platforms presented here enable continuous, highly sensitive, and localized rStO2 sensing at sites of interest in untethered, awake animal models. The use of RF-
based wireless power harvesting strategies and IR-based wireless data communication schemes allows deployment as subdermal oximetry implants for animal model studies without interruptions to natural behaviors. In addition, the mechanically compliant and biochemically inert designs avoid noticeable lesions or adverse immune responses, even when deployed in delicate regions of the brain.

The enabled capabilities for rStO₂ monitoring in targeted regions of deep tissues with millimeter-scale probing volumes complement those of conventional technologies (NIRS or cerebral oximeters, mostly for global or systemic rStO₂ measurements) that involve large form factors, limited probing depths (up to ~1 cm deep from the skin surface), and relatively large probing volumes (in the order of several to tens of cubic centimeters) (20, 38). These features are also distinct from those provided by recently developed thin, skin-mounted (pulse) oximetry platforms that exploit flexible mechanical designs and high-performance organic (39–41) or inorganic optoelectronic components (28, 29). The results create new possibilities for studying a broad range of O₂-mediated physiological and pathological processes in animal models, with potential for human translation. Examples include investigating function in specific regions of the brain using localized O₂ changes as surrogates of neural activity (32, 42–44), targeting of tumors via their association with hypoxia environments and low rStO₂ (3, 45), and postoperative (multisite) monitoring of tissue transplantation (e.g., flap reconstruction) where the early detection of compromised circulation in the form of low rStO₂ is critical (46, 47). For instance, these oximetry implants, either in the free-standing formats or mounted on conventional biopsy needles, can guide tumor targeting in specific regions by providing location-sensitive rStO₂ values in real time. This approach has the potential to reduce the chance of erroneous tumor targeting, especially for tumor tissues with very small sizes. Other direct or indirect O₂ measurement technologies for examining these processes involve physical tethers [e.g., bulky head stages (9) or electrodes with diameters of a few hundred micrometers (10, 48) for O₂ electrochemistry] or require specialized supporting equipment [e.g., EPR or BOLD-MRI equipped with magnetic fields of ~10 millitesla to multitesla (6); applicable only on anesthetized subjects]. In comparison, the devices demonstrated here favor rStO₂ measurements in freely moving animals without interruptions of their natural behaviors, especially in the context of social activities (10).

Fig. 4. Survey of location and tissue damage associated with wireless oximetry implants in the mouse brain. (A and B) Schematic and microscopic images of a representative mouse brain at the point of observation of tissue damage for lesion measurements. Scale bar, 2 mm (B). (C) Representative fluorescence images of horizontal striatum slices demonstrate lesion size (~360 µm × 240 µm) by immunohistochemical staining of neurons [Nissl, red; 4′,6-diamidino-2-phenylindole (DAPI), blue]. Scale bars, 100 µm. (D) 3D rendered microCT images of mice with battery-free, subdermal oximetry implants (highlighted in green color) in the brain. Scale bars, 3 mm.
The fabrication concepts and electronic designs introduced here can also be leveraged to enable implantable platforms with other functionalities. The programmable electronic designs allow μ-ILED modulation and data recording at high frequencies (~100 Hz), with potential applicability to tracking of heart rate and heart rate variability in small animal models (e.g., rats or mice, up to ~10 Hz) with optimization in signal-to-noise ratios and measurement schemes, an important physiological parameter and indicator of stress and other external stimuli in behavioral studies (49). Moreover, although the results shown here involve a single combination of two wavelengths (green and red), the designs are compatible with a variety of μ-ILEDs with other emission colors and with μ-IPDs that have spectral selective sensitivities, allowing ratiometric or photometric analysis of important biomarkers [e.g., Ca\textsuperscript{2+} or cancer biomarkers such as microRNA (50)] or physiological parameters. Other extended options include the integration of the oximeter probes with other functional modules for optogenetic modulation or microfluidic drug delivery. These multimodal systems with colocalization of stimuli and oxygenation detection could support unique capabilities in coupling the metabolism of specific tissue regions with external physiological or pathological challenges.

**MATERIALS AND METHODS**

**Fabrication of μ-IPDs**

The fabrication of μ-IPDs involved a series of photolithographic and etching steps on GaAs-based epitaxial materials (purchased from Masimo Semiconductor Inc.) with precise control over dopant levels in each layer [from top to bottom: n-type, Te-doped GaAs top contact layer (100 nm, >1 × 10\textsuperscript{19} cm\textsuperscript{-3}); n-type, Si-doped GaAs top contact layer (100 nm, 2 × 10\textsuperscript{18} cm\textsuperscript{-3}); n-type, Si-doped In\textsubscript{0.5}Ga\textsubscript{0.5}P window layer (25 nm, 2 × 10\textsuperscript{18} cm\textsuperscript{-3}); n-type, Si-doped GaAs emitter layer (100 nm, 2 × 10\textsuperscript{18} cm\textsuperscript{-3}); p-type, Zn-doped GaAs layer (2500 nm, 1 × 10\textsuperscript{17} cm\textsuperscript{-3}); p-type, Zn-doped Al\textsubscript{0.95}Ga\textsubscript{0.05}As back surface field layer (100 nm, 5 × 10\textsuperscript{18} cm\textsuperscript{-3}); p-type, Zn-doped GaAs bottom contact layer (300 nm, 5 × 10\textsuperscript{19} cm\textsuperscript{-3}); In\textsubscript{0.5}Ga\textsubscript{0.5}P window layer (700 nm); Al\textsubscript{0.95}Ga\textsubscript{0.05}As release layer (500 nm); and GaAs substrate/handling layer; see fig. S7]. Similar fabrication procedures appear in a recent report (18). First, photolithography with AZ nLOF 2070 negative tone photoresist (Integrated Micro Materials; spin-coated at 3000 rpm; developed with AZ MF 917 Developer), followed by electron beam evaporation of a bilayer of Cr/Au (10 nm/150 nm), defined the n-contacts. Using Cr/Au metal contacts as masks, a mixture of H\textsubscript{2}PO\textsubscript{4} (85 weight % (wt %) in H\textsubscript{2}O and 99.999% trace metal basis; Sigma-Aldrich), H\textsubscript{2}O\textsubscript{2} (30 wt % in H\textsubscript{2}O; ACS reagent, Sigma-Aldrich), and H\textsubscript{2}O with a volumetric ratio of 3:1:25 removed the exposed n-type, Te- and Si-doped GaAs layers. After defining the n-type regions of the μ-IPDs by photolithography with SPR v3.0 (MicroChem; spin-coated at 3000 rpm; developed with AZ MF 917 Developer), the n-doped In\textsubscript{0.5}Ga\textsubscript{0.5}P window layer and GaAs p-n junctions in the p-regions and other regions without photolithography were removed by HCl (37%; ACS reagent, Sigma-Aldrich)/H\textsubscript{2}PO\textsubscript{4} (1:1, v/v) and H\textsubscript{2}PO\textsubscript{4}/H\textsubscript{2}O\textsubscript{2}/H\textsubscript{2}O (3:1:25), respectively. Formation of the p-contacts (a bilayer of Cr/Au, 10 nm/150 nm) followed procedures similar to those for the n-contacts. Subsequent etching of heavily doped, p-contact layers (by H\textsubscript{2}PO\textsubscript{4}/H\textsubscript{2}O\textsubscript{2}/H\textsubscript{2}O\textsubscript{2}/H\textsubscript{2}O\textsubscript{2} (3:1:25) and intrinsic In\textsubscript{0.5}Ga\textsubscript{0.5}P window layers (by HCl) in selected areas (i.e., those uncovered by photocured SPR v3.0) yielded isolated arrays of μ-IPDs (each with a dimension of 100 μm × 100 μm). Last, undercut etching of the Al\textsubscript{0.95}Ga\textsubscript{0.05}As release layer by diluted hydrofluoric acid (HF) (HF/ethanol, 1:1.5) formed μ-IPDs in suspended configurations while tethered to the GaAs substrates via photopatterned, breakable polymer anchors.

**Fabrication of injectable sensing filaments of oximeters**

The fabrication followed procedures reported elsewhere (18) with some modifications. The fabrication of dual-layered, thin (width, ~380 μm) injectable filaments (fig. S1) started with lamination of a thin layer of PI (75 μm thick, Kapton, Fralock) onto a glass substrate (thickness, 1 mm) coated with PDMS (Sylgard 184, Dow Corning; part A/B, 10:1 in weight). A spin-cast PDMS layer (3000 rpm), after being fully cured, served as an adhesive layer between PI and glass. A PDMS stamp with relief structures enabled the microtransfer printing of a single μ-IPD from the growth substrate to the PI substrate coated with an adhesive layer with optimized formula (S1) [prebaked at 100°C for 7 min before the transfer printing and cured at 100°C under ultraviolet (UV) irradiation, ~10 mW cm\textsuperscript{-2}, for up to 1 hour]. With the μ-IPDs as the masking layers, reactive ion etching [March RIE; pressure, 200 mtorr; power, 100 W; oxygen gas, 20 standard cubic centimeters per minute (scm)] removed excessive adhesive materials and residues from the photoresists anchors. A 2-μm-thick photodefined layer of epoxy (SU-8 2002, MicroChem; spin-coated at 3000 rpm; developed with SU-8 Developer) then encapsulated the μ-IPD, leaving the p- and n-contact regions exposed. Subsequently, photolithography with AZ nLOF 2070 and liftoff with acetone defined the geometries of the metal interconnects (sputter-deposited layers of Cr/Au/Cu/Au/Cu/Au, 10/150/150/150/150/100 nm) and completed the fabrication of the μ-IPD layers. A photocured coating of epoxy (~7-μm-thick, SU-8 2007, MicroChem; spin-coated at 3000 rpm; developed with SU-8 Developer) encapsulated the μ-IPD layer before the fabrication of the metal interconnects (sputter-deposited layers of Cr/Au/Cu/Au/Cu/Au, 10/150/150/150/150/100 nm) for the μ-ILEDs. Laser-cutting (LPKF4 UV laser system) defined the shapes of the probes and microtransfer printing delivered the μ-ILEDs (green, CS27TR2227 from Cree Inc.; red, AERAX10 from Epistar) to the desired locations. An In-Ag alloy solder (Indalloy 290, Indium Corporation) enabled robust mechanical and electrical contacts between the pads of the μ-ILEDs and the sputtered interconnects by heating at 150°C for 2 min during the microtransfer printing process (18). The resulting injectable filaments with dual-layered structure were then integrated with the electronic modules by low-temperature reflow soldering or via connectors (503480-0500, Molex LLC) for devices powered by magnetic resonant coupling and batteries, respectively. Chemical vapor deposited layers of parylene (~14 μm) and an optional dip-coated layer of PDMS (thickness, ~10 μm) encapsulated the systems. Fabrication of injectable filaments/probes with other configurations followed similar procedures. The single-layered probes used metal interconnects for the μ-IPD and the μ-ILEDs sputter deposited on the same layer. The fabrication of probes with stretchable, serpentine-shaped interconnects included a spin-coated and cured layer of PI (thickness, ~5 μm) on top of the metal interconnects. This design placed the interconnect close to the neutral mechanical plane for strain reduction. Photolithographic patterning and reactive ion etching of the top PI layers in selected regions exposed the contacts.

**Fabrication of electronic modules of wireless, battery-free oximeters**

Fabrication of the electronic modules followed methods described elsewhere (25). Critical components included a μC (ATTiny84A, 3 × 3 mm package, Atmel), Schottky diodes for efficient power harvesting
In vivo rStO2 measurements on freely moving mice
In vivo rStO2 measurements of freely moving mice with battery-powered devices used C57Bl/6 mice (age, 12 weeks). The mice were group-housed before the implantation and thereafter individually housed. Implantation of battery-powered devices in the brains of mice followed reported procedures (18) except for the location (2 mm lateral and 4 mm deep to bregma). All procedures complied with the National Institutes of Health standards and were approved by the Animal Care and Use Committee of Washington University in Saint Louis. One day after recovery from surgery, freely moving, awake mice with implanted oximeters were transferred to a hypoxia chamber (Coy Laboratory Products, Grass Lake, MI) with precise control over FiO2 levels. The rStO2 measurements began with equilibrium at normoxia for about 5 min, followed by cycles of FiO2 (continuous changes in FiO2 between 21, 15, and 8%) in different sequences. Under hypoxia, mice showed less frequent movements and increased respiratory rates, with recovery to natural behavior and movements under normoxia. Data collection (25 Hz) and analysis followed procedures similar to those for anesthetized rats.

In vivo experiments on freely moving mice with battery-free, subdermally implantable oximeters used 30 to 40 g of CD1 IGS mice [Crl:CD1(ICR)] and involved procedures approved by the Institutional Animal Care and Use Committee (IACUC) of Northwestern University’s program for the human care and use of animals. The IACUC also inspects the animal facilities and investigator laboratories. Evaluation of the implanted devices was performed in compliance with Animal Welfare and Northwestern’s IACUC regulations. Sterilized devices (autoclaved) were implanted into the right striatum of anesthetized mice using methods described in a previous report (25). In the proximity of a mouse home cage equipped with a dual-loop primary antenna and connected to a commercial RF transmission system designed for optogenetics (Neurilux Inc.), the subdermally implanted oximeter remained operational in anesthetized and in awake, freely moving mice, with data streams consistent with those obtained benchtop experiments in terms of acquisition rate and signal quality. Analysis of the output timing on the bench and in the animal is shown in table S1.

Immunohistochemistry studies of brain tissues after device implantation
After a 4-week recovery period following brain surgery, mice with implanted oximeter devices were euthanized with pentobarbital sodium and intracardially perfused with 4% paraformaldehyde in PBS. The processing of sacrificed mouse brains included dissection, postfixation at 4°C for 24 hours, and cryoprotection in 0.1 M PBS (pH 7.4) containing 30% sucrose at 4°C for >24 hours. Brains were then cut into 30-µm sections and washed in PBS two to three times before being blocked in a blocking buffer (PBS containing 0.5% Triton X-100 and

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an average of $6.5 \times 10^6$ photons were launched from rectangular light sources, $240 \mu m \times 240 \mu m$ and $270 \mu m \times 220 \mu m$ for the red and green $\mu$-LEDs, respectively, with $120^\circ$ full divergence angle. The $\mu$-IPD has a surface area of $100 \mu m \times 100 \mu m$. With this stochastic photon propagation method, the estimated optical power/photoresponse was calculated for different rStO$_2$ at two wavelengths. The absorption coefficients [$\mu_a(\lambda)$] as a function of rStO$_2$ are given by the following equation (52, 53)

$$
\mu_a(\lambda) = \ln(10) \left( \frac{[Hb]_1}{M_w} (rStO_2 \cdot \epsilon_{HbO_2}(\lambda) + (1 - rStO_2) \cdot \epsilon_{Hb}(\lambda)) + W \mu_W(\lambda) \right)
$$

where [Hb]$_1$ is the total concentration of hemoglobin, $M_w$ is the molecular weight of hemoglobin (64,500 g mol$^{-1}$), $W$ is the water content (%), and $\mu_W(\lambda)$ is the absorption coefficient of water. The $\epsilon(\lambda)$ of HbO$_2$ and Hb, and $\mu_W(\lambda)$ are available from (27). Following from a previous report (32), the simulation set [Hb]$_1$ to 10 g liter$^{-1}$ (-0.1 mM) and W to 65% and swept rStO$_2$ from 10 to 60%, relevant to the range of calculated rStO$_2$, for in vivo experiments. For each $\mu$-LED, wavelength-averaged absorption coefficients [$\mu_{a-LED}(\lambda)$] accounted for dispersion in the emission spectra, using the following equation, where $\Delta_l$ is the full width at half maximum of the corresponding normalized LED emission spectrum ($\xi_{LED}$)

$$
\mu_{a-LED}(\lambda) = \frac{1}{\Delta_l} \int \mu_a(\lambda) \xi_{LED}(\lambda) d(\lambda)
$$

The scattering coefficient [$\mu_{s}(\lambda)$] at the dominant emission wavelength was calculated using the following equation (53)

$$
\mu_s(\lambda) = a \left( \frac{\lambda_{s0}}{\lambda_0} \right)^{-b} \frac{1}{1 - g}
$$

where $a$ is a scaling coefficient, $b$ is the scattering power, and $g$ is the anisotropy factor. For simulation in rodent’s brain ($a = 21.4$ cm$^{-1}$ and $b = 1.2$), the calculated $\mu_s(540$ nm$) = 195.2$ cm$^{-1}$ ($g = 0.89$) and $\mu_s(625$ nm$) = 163.2$ cm$^{-1}$ ($g = 0.90$). In comparison with $\mu_a(\lambda)$, which decreased with elevated rStO$_2$, $\mu_s(\lambda)$ remained constant in the course of the simulation.

To roughly estimate the contribution of photoresponses from light that passes directly from $\mu$-LEDs to the $\mu$-IPD (i.e., that flows through the encapsulation materials without being scattered by the surrounding tissues), additional simulations used the configuration outlined above but with $\mu_s(\lambda)$ set to $\sim 0.001$ cm$^{-1}$. Here, almost all light at the tissue/probe interface passed through the tissue without backscatter or propagation back to the $\mu$-IPD. This simulation method enabled quantified measurement of photoresponses that arise solely from direct light path. The averaged photoresponses (from six runs of simulation) indicated that the direct light path contribution is at least five orders of magnitude lower than that from backscattered light.

**rStO$_2$ data analysis and calculation**

Data analysis involved a commercial software package in MATLAB. Separation of the characteristic high and low output levels (in the form of ADC values) via a local minimal and maxima finding algorithm, followed by spline interpolation and down-sampling by a factor of 10, yielded data for two wavelengths. Optical densities as a function of time [OD($t$)] are defined as

$$
OD(\lambda, t) = -\ln \left( \frac{I_1(\lambda)}{I_0(\lambda)} \right)
$$

where $I_1(\lambda)$ is the time-dependent signal intensity and $I_0(\lambda)$ is the initial value, which were computed for both wavelengths ($\lambda_1$ and $\lambda_2$, green and red in this case). Other data processing of OD($\lambda$, $t$) included linear de-trending to remove the slow drifts and zero-lag digital Butterworth filtering with a low-pass cutoff frequency at 0.4 Hz to remove high-frequency noise (with respect to typical hemodynamics). Calculation of $\Delta[HbO_2]$ and $\Delta[Hb]$ followed from the modified Lambert-Beer law for diffusive media (54)

$$
\frac{\Delta[HbO_2](t)}{\Delta[Hb](t)} = \frac{1}{\rho} \left[ \frac{\epsilon_{HbO_2}(\lambda_1) \cdot DPF(\lambda_1)}{\epsilon_{Hb}(\lambda_1) \cdot DPF(\lambda_1)} \cdot \frac{\epsilon_{HbO_2}(\lambda_2) \cdot DPF(\lambda_2)}{\epsilon_{Hb}(\lambda_2) \cdot DPF(\lambda_2)} \right]^{-1} \times \frac{OD(\lambda_1, t)}{OD(\lambda_2, t)}
$$

where $\rho$ is the interoptode distance and DPF($\lambda$) is the differential path-length factor at the wavelength of interest, $\epsilon(\lambda)$ of HbO$_2$ and Hb are available from previous reports (27, 55). The DPF($\lambda$) were estimated assuming an infinite geometry and derived as the following equation (56), with $\mu_a(\lambda)$ and $\mu_s(\lambda)$ extrapolated from (57)

$$
DPF = \frac{3 \mu_s}{2 \sqrt{\mu_a}}
$$

Approximations of the initial [Hb]$_1$ (0.1 mM) and rStO$_2$ (at $t = 0$, 60%) (56) in the brain tissues of mice under ambient atmosphere enabled calculation of rStO$_2$ as a function of time for the in vivo experiments via the following equation

$$
rStO_2(t) = \frac{[HbO_2](t)}{[HbO_2](t) + [Hb](t)}
$$

$$
= \frac{[Hb]_1(t = 0) \cdot rStO_2(t = 0) + \Delta[HbO_2](t)}{([Hb]_1(t = 0) \cdot rStO_2(t = 0) + \Delta[HbO_2](t)) + ([Hb]_1(t = 0) \cdot (1 - rStO_2(t = 0)) + \Delta[Hb](t))}
$$

**MicroCT imaging**

Mice with subdermally implanted oximeters in the striatum were anesthetized in an induction chamber with 3% isoflurane in O$_2$ and
transferred to a dedicated imaging bed with isoflurane delivered via nose cone at 1 to 2%. The animals were then placed in the prone position with head immobilized with ear and tooth bars, and respiratory signals were monitored using a digital system (Mediso USA, Boston, MA). A preclinical microCT imaging system (nanoScan PET/CT, Mediso USA, Boston, MA) acquired images with the following parameters: x×2.2 magnification, <60-μm focal spot, 1×1 binning with 720 projection views over a full circle by using 70 kVp/520 μA with an exposure time of 300 ms. The projection data were reconstructed with a voxel size of 34 μm using filtered (Butterworth filter) back-projection software from Mediso. The reconstructed data were visualized and segmented in Amira 6.5 (FEI, Houston, TX).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/3/eaaw0873/DC1

Fig. S1. Scheme of the fabrication steps of the dual-layered wireless, battery-free oximeters.

Fig. S2. Scheme of wireless oximeters with different designs of injectable filaments.

Fig. S3. Optical images of the tip end of injectable filaments of wireless oximeters.

Fig. S4. Photographs of wireless oximeters with battery-powered electronic modules.

Fig. S5. Photograph of a battery-free, fully implantable, wireless oximeter on a balance.

Fig. S6. Ratio of extinction coefficients of HbO2 and Hb.

Fig. S7. Scheme of the epidermal stack of GaAs wafers used for the fabrication of μ-IPDs.

Fig. S8. Scheme and pseudocolored SEM image of GaAs-based μ-IPDs.

Fig. S9. Characterizations of materials design to integrated optoelectronic platforms.

Fig. S10. Monte Carlo simulation results.

Fig. S11. Monte Carlo simulation results.

Fig. S12. Scheme of the experimental arena circumflexed with antenna for wireless power supply.

Fig. S13. Transmittance spectra of mouse scalp collected by a fiber optic spectrometer.

Fig. S14. Flux calculations in output signals (ΔQ/Δt) of the wireless, battery-free oximeters over time.

Fig. S15. Absorption spectra of five artificial blood solutions with various combinations of oxyhemoglobin, deoxyhemoglobin, and methHb.

Fig. S16. Raw data of rStO2 collected by wireless, battery-powered oximetry implants in the tissue region near femoral artery of anesthetized rats.

Fig. S17. Surgical steps of the subdermal implantation of wireless, battery-free oximeters in mouse brain.

Fig. S18. Photographs of freely moving mice with brain-implanted oximeter filaments with connectors for the integration with battery-powered electronics.

Fig. S19. Weight changes of three mice after subdermal brain surgery with wireless, battery-free oximeter implants.

Table S1. Data transmission of the wireless, battery-free oximeters before and after subdermal implantation.

Movie S1. A wireless, battery-free, fully implantable oximeter with illuminating μ-LEDs.

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