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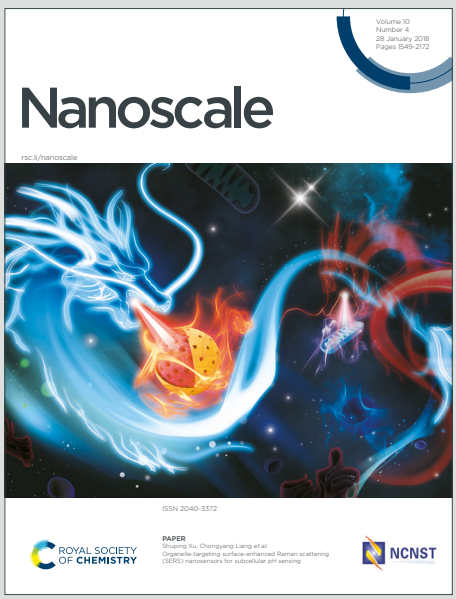

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Flexible and Fully Implantable Upconversion Device for Wireless Optogenetic Stimulation of Spinal Cord in Behaving Animals

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Abstract

Wireless optogenetics based on upconversion technique recently provides an effective and interference-free alternative for remote brain stimulation and inhibition in behaving animals, which is of great promises for neuroscience research. However, more versatile upconversion devices are yet implemented for neural tissues other than the brain. In this study, a flexible and fully implantable upconversion device is developed for epidural spinal cord stimulation. The upconversion device is fabricated via a straightforward two-step heat-pulling process using biocompatible thermoplastic polypropylene as a backbone, which is mixed with upconversion nanoparticles (UCNPs) to form a flexible optrode device that converts near infrared (NIR) irradiation to visible lights for optogenetic manipulation of spinal cord tissues. In this system, the flexible upconversion device is fully-implantable within the rigid spine structure, and shows excellent long-term biocompatibility even after four month experiment. In anesthetized mice, the UCNPs device implanted at L4 vertebra can be used to reliably evoke hindlimb muscular activity upon NIR triggering. In behaving mice, neural modulation by the same UCNPs devices effectively inhibits the animals' movement as a result of remote spinal cord stimulation. We believe that the flexible upconversion device provides new possibilities for wireless neural modulation in spinal cord tissues, and will become a valuable supplement to the current toolsets of upconversion based wireless optogenetics.

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Introduction

In the nervous system, the spinal cord plays fundamental role to transmit neural signal and control the motor function of vertebrate animals.[1] Effective manipulation of neural signals in the spinal cord is important in neuroscience research and clinical practice for treating disorders related to strokes, pain, and neurotrauma.[2-6] Clinically, electrical epidural stimulation is applied in the spinal cord for alleviation of pain.[7] Spinal cord epidural stimulation is also used to mimic the neural signals from the brain to initiate locomotion.[3, 8] The traditional electrical stimulation lacks activation selectivity and is poor in spatial resolution. While optogenetics could achieve better selective and spatial-temporal manipulation of neural activity,[9, 10] few reports have documented optogenetic stimulation of spinal cord tissue in live animals, partially due to the complicated structure of spinal cord and the bulky devices for effective light delivery through tissues and bones.[11-13] The spinal cord shielded in vertebras is vastly different in sizes, shapes, and tissues stiffness depending on the anatomical position. Also, animal's constant movement further hinders the delivery of light.[11]

To conduct wireless optogenetics in spinal cord tissues, the latest feasible solutions are based on radio-frequency powered LEDs [14] or a stretchable optoelectronic system.[15] In both cases, the implanted devices require sophisticated fabrication and only work with a special powering system, which is costly and may not be conveniently accessible to researchers. Recently, a simple and low-cost wireless optogenetic system was demonstratted by using near infrared (NIR) and upconversion technology.[16, 17] In this system, upconversion nanoparticles (UCNPs) are packaged as a microscale optrode device, which can be remotely triggered by NIR to stimulate various brain regions in behaving rodents. The system combines two critical features to implement an all-optical wireless optogenetics solution: NIR of 700~1100 nm in wavelength

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can effectively penetrate tissues because of low in-tissue scattering, which can be further reduced by applying NIR of longer wavelength [18]; the UCNPs convert low-energy NIR photons to high-energy photons within visible spectrum.[19-23] However, the feasibility and performance of an all-optical solution based on fully implantable UCNP-based device and wireless NIR triggering system for spinal cord stimulaiton of behaving rodents remains unexplored.

Here, using thermoplastic biocompatible polypropylene (PP) as backbone, we fabricated the flexible and fully implantable UCNP-PP-optrode (or UCNP-device) for effective epidural spinal cord stimulation *in vivo*. The two-step fabrication process is straightforward and highly adaptable. In mice, the UCNP-PP-optrode was implanted on the dura mater of the targeted spinal cord transfected with AAV6-Syn-ChR2-EYFP virus for optogenetics stimulation. In anesthetized mice, UCNP-PP-optrode triggered by NIR laser evoked significant hindlimb muscular activity which is recorded by electromyography (EMG), indicating successful spinal cord activation to transmit neural signal to evoke muscular activity. Further, the functional UCNP-PP-optrode was applied to intervene movement of freely-moving mice in an open field test. Upon NIR laser projected to the optrode region, the exploratory activity was effectively decreased, demonstrating the feasibility of applying such technique on behaving mice in neuroscience research. Even after implanted for up to four months, our functional optrode induced little inflammatory response in the surrounding spinal cord tissues, showing excellent long-term biocompatibility *in vivo*. Taken together, our UCNP-PP-optrode is a promising technique for effective wireless optogenetics manipulation in spinal cord for long-term behavior test *in vivo*.

Results

Fabrication and implantation of the flexible UCNP-PP-optrode

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The fabrication of UCNP-PP-optrode is a two-step process. PP tube was chosen as substrate material because of its superior biocompatibility, moderate mechanical strength, and thermoplasticity. UCNPs dispersed in cyclohexane were loaded into the hollow PP tube and dried on the inner wall after solvent evaporation. Afterwards, heat-pulling was implemented to pull the UCNP-containing PP tube into tapered fiber (Figure 1a), and the fiber was cut into sections of 2 mm in length. The fabricated UCNP-PP-optrode packaged the UCNPs into its structure, which guarantees no direct contact between the UCNPs and the cells to improve long-term biocompatibility. Also, the functional optrode measured in ~500 μm in diameter and weighted less than 1 mg is compact enough to be implanted, and causes almost no obstructive effect and tissue damage after recovery.

Four weeks prior to UCNP-device implantation, the dorsal horn of the L4 lumbar vertebra of a mouse (ICR strain) was injected with AAV6-Syn-ChR2-EYFP viruses to express light-sensitive channelrhodopsin (ChR2) in neurons (specified in *Experimental sections*). For surgical implantation, the UCNP-PP-optrode was placed at and pressed into the hole drilled for virus delivery, and the surgical site was sealed with a patch of transparent polydimethylsiloxane (PDMS) as artificial skin (Figure 1b). The implanted mouse was allowed to recover for one week before any experiments.

Optical epidural spinal cord stimulation

The transparent stretchable PDMS patch provided a window to allow NIR transmission to activate the implanted UCNP-device for neural stimulation (Figure 2a). Upon excited by 980 nm laser (20 mW mm^{-2}), the implanted UCNP-PP-optrode was activated, and the emission of blue light can be readily captured by the *in vivo* imaging system, as shown in Figure 2b. The X-ray

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scanning revealed intact spinal structure after the surgical operation. The merged fluorescent and X-ray images indicated the precise placement of the UCNP-PP-optrode at L4 spinal vertebra.

As the L4 lumbar region is involved in the control of hindlimb motor function, an EMG recording electrode was inserted into the hindlimb gastrocnemius muscle of an anesthetized ICR mouse to record evoked muscular electrical signal (details in *Experimental sections*) in response to NIR irradiation. Upon pulsed NIR stimulation (20 mW mm^{-2} for 50 ms), significant muscular activity was evoked in ChR2-transfected mice implanted with functional UCNP-device, showing a synchronized pattern with the NIR pulses (Figure 2c & d). Similar muscular activity was not observed in the control group, ChR2-transfected mice and implanted with a dummy optrode that contained no UCNPs, indicating successful muscular innervation in the functionally intact neural circuitry from the lumbar spinal cord to hindlimb gastrocnemius muscle by the NIR excited UCNP-PP-optrode. The temporal correlation between NIR stimulation and gastrocnemius muscle activity was characterized by post stimulation time histogram (PSTH). In the PSTH, pulses of NIR trigger (20 mW mm^{-2} for 50 ms) evoked significant muscular activities for ~80 ms, and the delay between stimulation and evoked EMG signal is around 90 ms (Figure 2e).

Performance of UCNP-PP-optrode for evoking muscular activity

The level of evoked muscular activities is a vital indicator for assessing the performance of epidural spinal cord stimulation. As the NIR irradiation intensity was raised from 8 to 24 mW mm^{-2}, the integrated EMG (iEMG) showed an increasing pattern from ~0.5 to ~2 mV ms (Figure 3a). However, in the control group (ChR2-mice with a dummy-optrode), NIR irradiation as strong as 24 mW mm^{-2} did not evoke any detectable activities (Figure 3a). Similar pattern was also observed in the RMS peak value (Figure 3b). The RMS peak value of properly stimulated

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mice was significantly higher than that recorded from the control animals even at the lowest NIR intensity of 8 mW mm^{-2}.

The activation of spinal cord neurons was further characterized by immunostaining for activated neurons following NIR irradiation (20 mW mm^{-2} power density, 0.5 Hz frequency, 50 ms pulse width, 20 pulses every 5 minutes, 30 minutes duration) to trigger the gastrocnemius muscle activity. After the experiment, mice were perfused and fixed to harvest the stimulated spinal cord tissue. Around the regions surrounding the UCNP-PP-optrode, strong expression of c-fos, which is a marker for cell activation, was found to be co-localized with ChR2-$EYFP^{+}$ neurons as far as ~300 μm from the UCNP-device (Figure 3c), confirming that the innervation of the gastrocnemius muscle was originated from the excitation of the associated spinal cord neurons stimulated by NIR-triggered UCNP-pp-optrode. The statistical analysis showed that 29.29±1.68% cells around the UCNP-device were successfully transfected to express ChR2 and 64.79±4.82% of the transfected neurons were activated by NIR-triggered UCNP-pp-optrode (Figure 3d & e).

Inhibition of movement in open field test

The utility of the UCNP-PP-optrode was then tested in freely-moving mice to see if the upconversion-based wireless stimulation of epidural spininal cord can intervene the movement in behaving animals. ChR2-transfected mice implanted with either a functional or a dummy optrode was housed in a open-filed equiped with a custom-bulit robotic NIR laser projection system for flexbile remote NIR irradiation.[16, 24] The laser was configured to aim at the surgical region throughout the open field test (Figure 4a; also see Supplementary Video).[25, 26] In this experiment, the mouse was first allowed to freely explore the environment for 5 minutes within the apparutus. Over the four-minute experimental period, the laser projection system automatically

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tracked the movement of the mouse, and projected NIR laser (20 mW mm^{-2} power density, 1 Hz frequency, 50 ms pulse width) precisely at the surgical region in the later two minutes of each trial (Figure 4b). For mice with successful spinal cord stimulation by upconversion-based wireless optogenetics, the movement of mice with a functional UNCP-device was dramatically inhibited, and showed a significant shorter moving trajectory reduced from the baseline acivity when the stimulation NIR was turned off (in the first two minutes, Figure 4c, d), but the functional UCNP-pp-optrode did not affect the maximum movement speed in the animlas (Figure 4e). Such behaviroal interferece was not resulted from any temperature fluctuation induced by NIR irraditation (Figure S2), and was not observed in ChR2-transfected mice implanted with a dummy optrode (no UCNPs, Figure 4d, e), emphasizing the critical importance of upconversion in this setup. The open field test further demonstrated successful muscular intervention that affected the animal movement by using the NIR-triggered UCNP-PP-optrode, and shoed a great potential of the upconversion-based wirelss optogenetics as an useful technique for spinal cord stimulation in freely-moving animals.

Long-term biocompatibility of the UCNP-pp-optrode

As a promising wireless optogenetics technique for behaving rodents, the biocompatibility is of great concern. To test the long-term biocompatibility, a UCNP-PP-optrode was implanted and maintained in the animal for four months. Thereafter, the mice were sacrificed, and its spinal cord tissues surrounding the optrode were harvested for immunohistochemical analysis. Astrocyte ($GFAP^+$) and microglia ($Iba\text{-}1^+$) were stained in the tissue slices from animals with or without an UCNP-device implantation. Only a small fraction of astrocyte activation surrounding the UCNP-device was observed due to minor surgical damage when compared to results from the intact spinal cord tissues (Figure 5a, b). The UCNP-pp-optrode was well tolerated by the

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animal, and no noticeable inflammatory response was found in the spinal cord tissues, which was confirmed by the quantitative analysis of microglials and astrocytes in the intact and optrode-implanted spinal cord tissues (Figure 5c & d).

Discussion

In this study, a wireless and fully implantable device was developed for wireless optogenetic stimulation of spinal cord in behaving animals. The device is based on a flexible UCNP-PP-optrode which can be excited by remotely applied NIR laser irradiation. Unlike traditional optogenetic experiments, in which visible light (400~600 nm) are used neural manipulation, NIR light has much deeper tissue penetration capability, which can be utilized for optogenetic activation of deep brain tissues when combine with upconversion techique.[17, 18] The UCNP-PP-optrode can effectively convert tissue-penetrating NIR light to blue visible light (~488 nm) for stimulating ChR2-expressing neurons, providing a convenient and highly efficient solution for wireless optogenetics manipulation in freely moving rodent animals. The upconversion efficiency is generally around 3~4%,[24] the blue emission from the UCNP-pp-optrodes is around 12.5 mW, given an NIR irradiation intensity of 20 mW mm^{-2} (~400 mW). Such intensity renders an activation range around 300 μm from the UCNP-device, as estimated from the expression c-fos after consistent stimulation of relevant neurons (Figure 3c). The UCNP-device is flexible and compact (~500 μm diameter and <1 mg weight), and can accommodate delicate experiments in small animals, such as mice and rats, without causing any significant damage to the spinal cord tissue, especially in behaving animals (Figure 2b). Thus, this study demonstrates an all-optical alternative based on upconversion technique for long-term wireless optogenetic applications *in vivo*.

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In spinal cord tissues, a central pattern generators (CPGs) is a network of neurons and other cells to produce rhythmic signal for controlling locomotion.[27] It was shown that when epidural stimulation was successfully delivered to spinal cord to evoke CPGs, the coordinated patterns of locomotion (e.g., stepping) would be reproduced even without the input signal from the supraspinal level.[28, 29] Traditionally, the electrical stimulation system typically requires serval components, including electrode arrays, wire connectors, and current/voltage pulse generator coupled with external power source.[3, 30, 31] The bulky hardware and tethered cables would substantially interfere the movement of experimental animals, which potentially lead to false positive/negative or unexplainable results in many behavioral tests. Towards a solution to these problems, different strategies of wireless optogenetics have been implemented.[14, 15] In our upconversion-based and all-optical system, the core is a microscale UCNP-device packaged in flexible PP material, which was specially designed to accommodate delicate implantation to the spinal cord tissue without any causing noticeable tissue damage and heavy surgical burden to the animals. The used UCNP-pp-optrode was shielded by a patch of transparent PDMS and remotely triggered by NIR laser irradiation. In this study, we have observed that the intensity of evoked hindlimb muscular activities can be fine-tuned by changing the NIR irradiation power (Figure 3a & b), as a result of varying blue emission for exciting CHR2 expression neurons. Significant iEMG signal was recorded at a NIR irradiation intensity of 12 mW mm^{-2} and above (Figure 3a). Similar signals was not observed in the control animals, AAV-infected animals implanted with a dummy device that does not contain any UCNPs, emphasizing the critical importance of upconversion in this setup, and also ruling out the interference by other factors, including surgical pain, skin heating, and virus infection, etc.. For more useful animal experiments involving epidural spinal cord stimulation, the UCNP-PP-optrode was also tested in behaving

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mice by using a customized laser projection system, which can automatically and efficiently place the NIR stimulation signal on the targeted spot of a mouse/rat (Figure 4d), demonstrating the feasibility and effectiveness in controlling the locomotive behavior in freely moving mice by wireless optogenetic stimulation of spinal cord tissue using the flexible and fully implantable upconversion device.

Another advantage of the demonstrated UCNP-device is the superior biocompatibility, as PP is an organic thermoplastic material well tolerated by spinal cord tissues.[32] Traditional metal electrodes would cause significant immune response after *in vivo* implantation, which can deteriorate stimulation efficiency gradually in behaving animals.[33] Using PP as backbone material to package the potentially cytotoxic UCNPs [34], the implant is safe to be kept inside the spinal cord for up to four months and induces almost negligible long-term immune responses (Figure 5). Because the UCNPs were completed sealed in the PP and isolated from cells and *in vivo* microenvironment, the performance of the device was not compromised after four-month-implantation, showing a potential for even longer behavioral experiments.

Conclusion

In this study, a flexible and fully implantable UCNP-device was described for epidural spinal cord stimulation upon NIR laser irradiation in both anesthetized and freely-moving rodent animals. The functional UCNP-device efficiently evoked hindlimb muscular activities as recorded by EMG, as a result of proper stimulation of the L4 vertebra lumbar of spinal cord. In an open field test, the locomotive activity of ChR2-transfected mouse was also successfully interfered by remotely applied NIR irradiation when implanted with the UCNP-device. We believe that the flexible UCNP-device provides new possibilities for wireless neural modulation

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in spinal cord tissues, and will become a valuable supplement to the current toolsets of upconversion-based wireless optogenetics.

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Experimental sections

Materials

The chemicals used in this study were purchased from Sigma Aldrich. For the materials related to experiments involving animals, the reagents were mainly purchased from Thermo Fisher Scientific, unless otherwise specified.

Synthesis of UCNP

The UCNPs were synthesized following a protocol stated in our previous report. [24] Briefly, the procedures involve two main processes, growth of the core and coating of the shells. For the synthesis of core, an aqueous solution (2 mL) containing $Y(Ac)_3$, $Yb(Ac)_3$, and $Er(Ac)_3$ (0.2 M each) was mixed with oleic acid (3 mL) and 1-octadecene (7 mL), and the mixture was heated to 150 ℃ for 30 minutes followed by cooling down to 50 ℃. Afterwards, a methanol solution (5 mL) with NH_4F (1.6 mmol) and NaOH (1 mmol) was added and mixed by stirring for 30 minutes. With methanol totally evaporated, the mixture was further heated to 300 ℃ under argon for 90 minutes and cooled down to room temperature. The synthetic cores were precipitated by adding ethanol and collected by centrifugation. The cores were washed in methanol and ethanol, and finally dispersed in cyclohexane. For the shell coating, oleic acid (3 mL) and 1-octadecene (7 mL) were added to an aqueous $Y(Ac)_3$ solution (0.2 M, 2 mL), and the mixture was heated to 150 ℃ for 30 minutes and cooled down to 50 ℃. Thereafter, cores dispersed in cyclohexane (4 mL) and a methanol solution (5 mL) containing NH_4F (1.6 mmol) and NaOH (1 mmol) were added and stirred at 50 ℃ for 30 minutes. The mixture was further heated to 300 ℃ under argon for 90 minutes and cooled down to room temperature. The coated particles were again precipitated, collected, washed, and dispersed in the aforementioned manner.

Fabrication of UCNP-device

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The PP tube (5 mm outer diameter and 4.5 mm inner diameter) was loaded with cyclohexane containing dispersed UCNPs at its center along length by microinjector. After the solvent evaporated, UCNPs were dried on the inner wall of the tube. The UCNP-loaded PP tube was heated to melt at ~140℃ by heater, and subsequently pulled from both ends in opposite directions, resulting in two separate tapered fibers (0.5 mm diameter). The loading process and heating process could be repeated several times to guarantee enough UCNPs were packaged into the fiber. Finally, the fiber containing UCNPs was cut into 2 mm sections, which is ready for implant.

Animals

ICR mice (6 ~ 8 weeks, 20 ~ 25 g in weight) were used in this study. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of City University of Hong Kong and approved by the Animal Ethics Committee of City University of Hong Kong. Animal were housed in facilities in campus, following standard animal guideline.

Optogenetics virus delivery

The vector (AAV6-Syn-ChR2-EYFP) was acquired from the Vector Core at the University of North Carolina at Chapel Hill. Prior to anesthesia, atropine sulfate (0.05 mg kg^{-1}) was administered intraperitoneally to inhibit tracheal secretions. Mice were anesthetized with sodium pentobarbital (50 mg kg^{-1}) throughout the whole surgical operations. The mice were then mounted on a stereotactic frame. The mice body temperature was maintained at ~37℃ by a heating pad placed beneath. An incision was made in the back skin with local anesthetics (Xylocaine, 2%) applied around the opening. A hole (0.5 mm diameter) was drilled at the lumbar vertebra (L4) to get access to the dorsal horn for virus injectoin. The size of the hole was carefully controlled to match the diameter of the UCNP-pp-optrode for later device implantation

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and fixation. The virus was injected into the left dorsal horn at a rate of 0.02 μl min^{-1} (0.5 μl total volume) using a glass micropipette connected to a syringe pump. After withdrawing the injection micropipette, the injection site was cleaned with sterile saline, and the skin was sutured. The mice were allowed to recover for four weeks before proceeding to the following procedures.

UCNP-device implantation

The implantation of UCNP-PP-optrode was performed four weeks after virus injection. During the surgical operation, the mice were anesthetized. Local anesthetics (xylocaine, 2%) was applied to the region used for virus injection, and an incision was again made around that region for UCNP-pp-optrode implantation. Afterwards, the hole for optogenetics virus injection was found and cleared with driller, where the functional optrode was slowly inserted into with fine surgical tweezers. The UCNP-pp-optrode (~500 μm diameter and 2 mm length) was pressed against the hole (~500 μm diameter) and got stuck there to prevent dislocation, and the device was further covered and fixed by the surrounding muscle tissues. After surgery, a PDMS artificial skin was sutured on the surgical site covering the optrode implantation. The mice were allowed for recovery for one week before further experiments.

Electromyography recording

EMG recording was acquired via a three-electrode scheme: the recording electrode was inserted in the gastrocnemius muscle; the reference electrode was placed in achilles tendon; the ground electrode was inserted subcutaneously into the mouse back. EMG electrodes were connected to the preamplifier (GRASS INSTRUMENT) with a 30-10 kHz filter applied for the EMG signals. A digitizer (Molecular Devices) was used to record amplified EMG signal and control the timing of NIR laser (Kaipulin Laser Technology). All EMG signal and corresponding timing of NIR laser were analyzed using Clampfit 10.7 (Molecular Devices).

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Immunohistochemistry

Mice were anesthetized with pentobarbital and transcardially perfused with chilled phosphate-buffered saline (PBS) and paraformaldehyde (PFA; 4% in PBS). The spinal cord was harvested and further fixed in 4% PFA for 2 day at 4ºC. Samples were then bathed in 30% sucrose solution (prepared in PBS) until settled to the bottom. The tissues were then cut into slices (100 μm thickness) by frozen sectioning (Thermo Scientific) and mounted on glass slides.

All slices were thoroughly rinsed with PBS and blocked with 4% BSA in Tris-buffered saline containing 0.25% Triton X-100 (TBS-T) overnight at 4ºC. The slices were then incubated with primary antibodies overnight at 4ºC followed by thorough rinsing with TBS-T. Secondary antibodies were applied for 1 hour at room temperature followed by thorough rinsing with TBS-T. The stained spinal cord slices were mounted for imaging. Samples were imaged using confocal microscope equipped with a 40× water-immersion objective (Leica Microsystems). The primary antibodies used in this study included rabbit anti-c-fos (Abcam, ab190289, 1:2000 dilution), rabbit anti-GFAP (Millipore, AB5804, 1:1000 dilution), and goat anti-Iba-1 (Abcam, ab5076, 1:500 dilution).

Live animal imaging

In vivo imaging of live animal was carried out using a multi-modal imaging system (Bruker). The mice were anesthetized by injection of sodium pentobarbital (50 mg kg^{-1}). A 980 nm laser generator was custom-installed within the animal imager for exciting UCNP-PP-optrode from the above. A 480 nm emission filter was used for capturing emitting light from excited optrodes.

Laser projection system for behaving test

To enable precise and real-time laser projection to the targeted region, a customized tracking system was built as previously reported.[35] In the system, a field chamber was used to house the

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animal during an experiment and a camera was placed above the chamber to track targeted the moving animal and to identify the targeted region. The laser projection component is comprised of a robotic arm driven by two digital motors and a collimated NIR laser source. Before any experiment, a calibration was carried out to link the spatial coordinates of the chamber with angle setting of the two digital motors for later NIR project. And a set of parameters for tracking the artificial PDMS skin (covering functional UCNP-pp-optrode) was also acquired and tested to enable automatic aiming with a feedback from the camera recording. During an experiment, the system was able to project the NIR laser directly on the surgical site of a freely moving animal in real-time. The animals were allowed to explore the environment for a certain period before any behavioral experiments, which included two sections: a two-minute section without NIR irradiation and another two-minute section with NIR irradiation.

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Author Contributions

P.S. conceived the project and supervised the study. Y.W. performed experiments and analyzed data. X.C. and F.W. provided the upconversion particles. K.X., H.Y., and X.L. helped on different aspects of the experiments. Y.W., K.X., and P.S. wrote the manuscript.

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Competing financial interests

The authors declare no competing financial interests.

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Figures

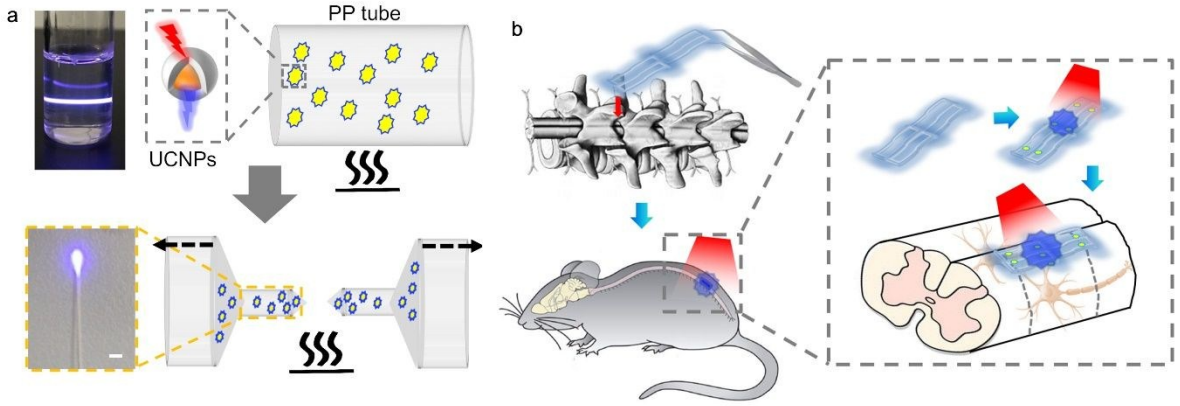

Figure 1. Diagram of fabrication and implantation of the UCNP-PP-optrode. (a) Diagram of UCNP-PP-optrode fabrication. In the upper panel, the PP tube loaded with UCNPs was melted. In the lower panel, the melted PP tube was pulled to form fiber-like functional optrode. Scale bar, 2 mm. (b) Diagram of implantation of the UCNP-PP-optrode at the spinal cord of mouse by minimal invasive surgery. The mouse was first transfected with optogenetics virus at the L4 lumbar vertebra. After recovery, the transfected mouse was implanted with our UCNP-PP-optrode at the transfected region, and the surgical site was covered with artificial PDMS skin to allow efficient NIR delivery after surgery.

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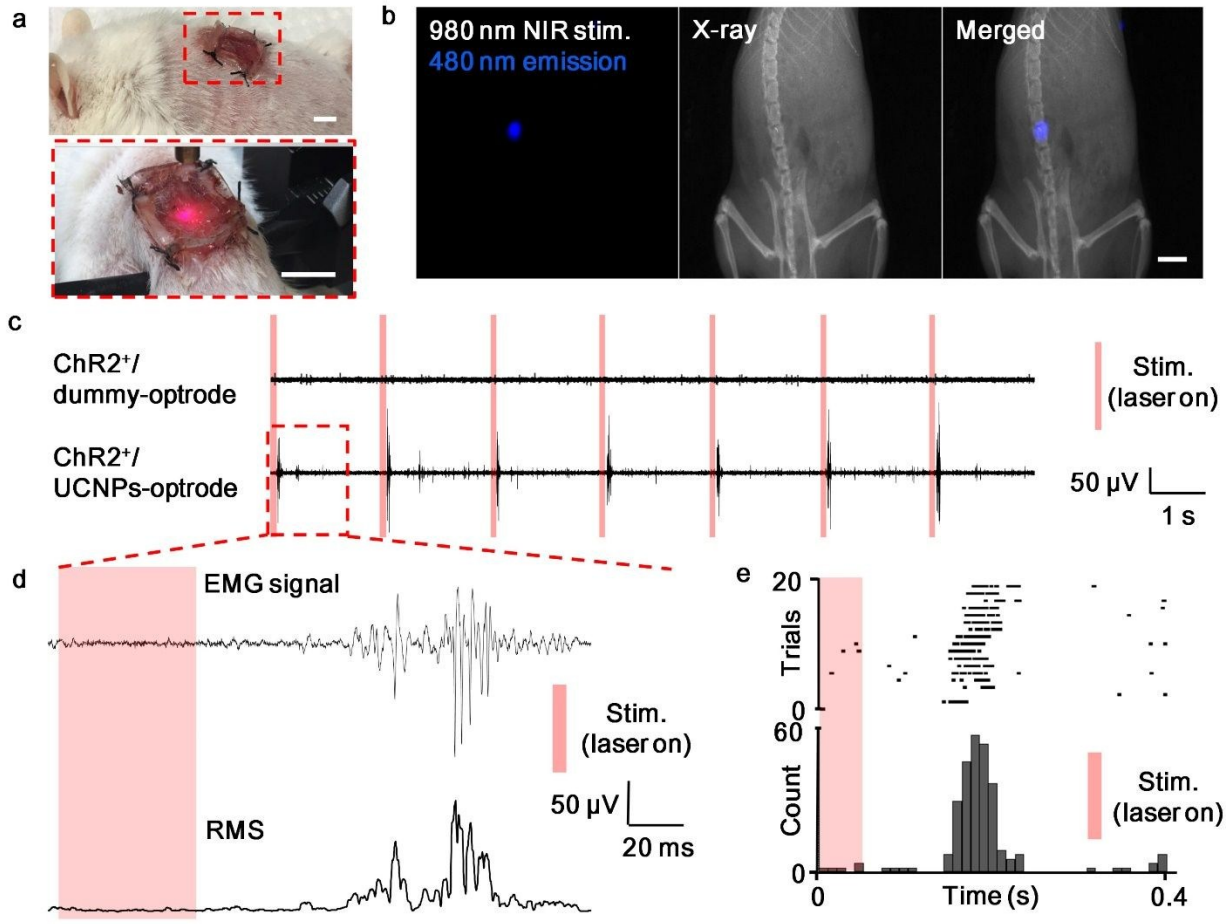

Figure 2. Optical epidural spinal cord stimulation using UCNP-PP-optrode and NIR irradiation. (a) Photogram of a mouse implanted with the UCNP-PP-optrode. The surgical region was shielded by artificial PDMS skin. Scale bar, 1 cm. (b) Mice implanted with UCNP-PP-optrode was stimulated by NIR laser (left). An x-ray image (middle) was also taken to confirm the correct implantation location (right) using an *in vivo* imager. Scale bar, 2 cm. (c) Representative EMG recordings of ChR2-transfected mice implanted with a dummy optrode or UCNP-PP-optrode in response to NIR laser stimulation (20mW mm^{-2} power density, 0.5 Hz frequency, 50 ms pulse width). (d) Typical EMG recording of ChR2-transfected mice implanted with UCNP-pp-optrode in response to NIR laser stimulation (20mW mm^{-2} power density, 0.5 Hz frequency, 50 ms pulse width). (e) Raster plot and PSTH (10 ms bin^{-1}, 20 trials) show synchronous temporal correlation between evoked hindlimb muscular activity and NIR laser stimulation (20mW mm^{-2} power density, 0.5 Hz frequency, 50 ms pulse width) in transfected mice implanted with functional optrode.

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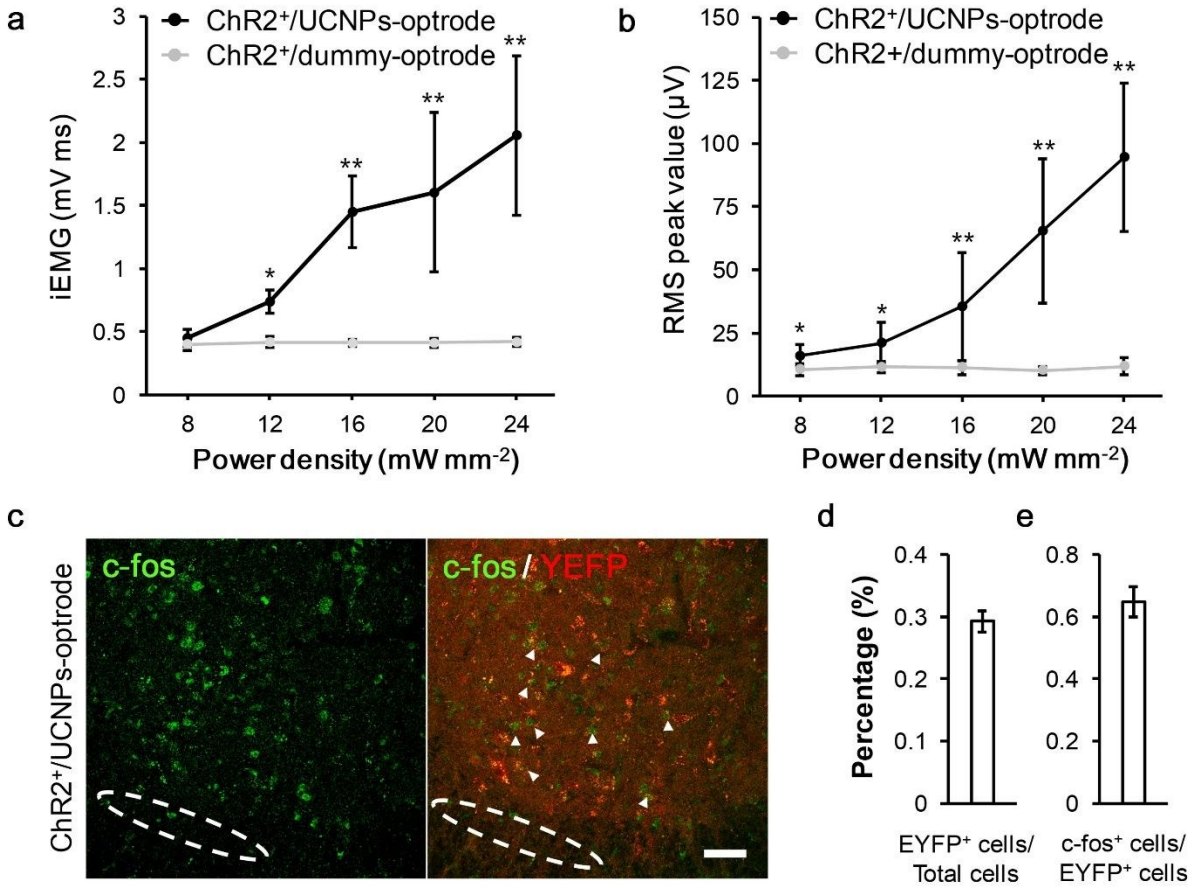

Figure 3. Performance of UCNP-PP-optrode for spinal cord stimulation to activate muscular activities. (a-b) Evoked iEMG (a) and corresponding RMS peak value (b) in response to different NIR laser intensity settings (200 ms following stimulation; n=6). Bin size of RMS is 1 ms. * for $P<0.05$ and ** for $P<0.005$ by student t-test. (c) Immunostaining of transfected mouse spinal cord tissue surrounding the UCNP-optrode showed the activated neurons (c-fos$^+$; left panel) around UCNP-optrode (white dashed circle) highly overlaid with ChR2-EYFP$^+$ lumbar neurons (pointed by white arrow heads in right panel) following NIR laser stimulation (20 mW mm^{-2} power density, 50 ms pulse width, 0.5 Hz frequency, 20 pulses every 5 minutes, 30 minutes duration). Scale bar, 50 μm. (d) Efficiency of transfection efficiency by AAV injection (n=5). (e) Efficiency of activated ChR2$^+$ neurons activation in mice implanted with a UCNP-pp-optrode (n=5). For all panels, the error bars indicate standard error.

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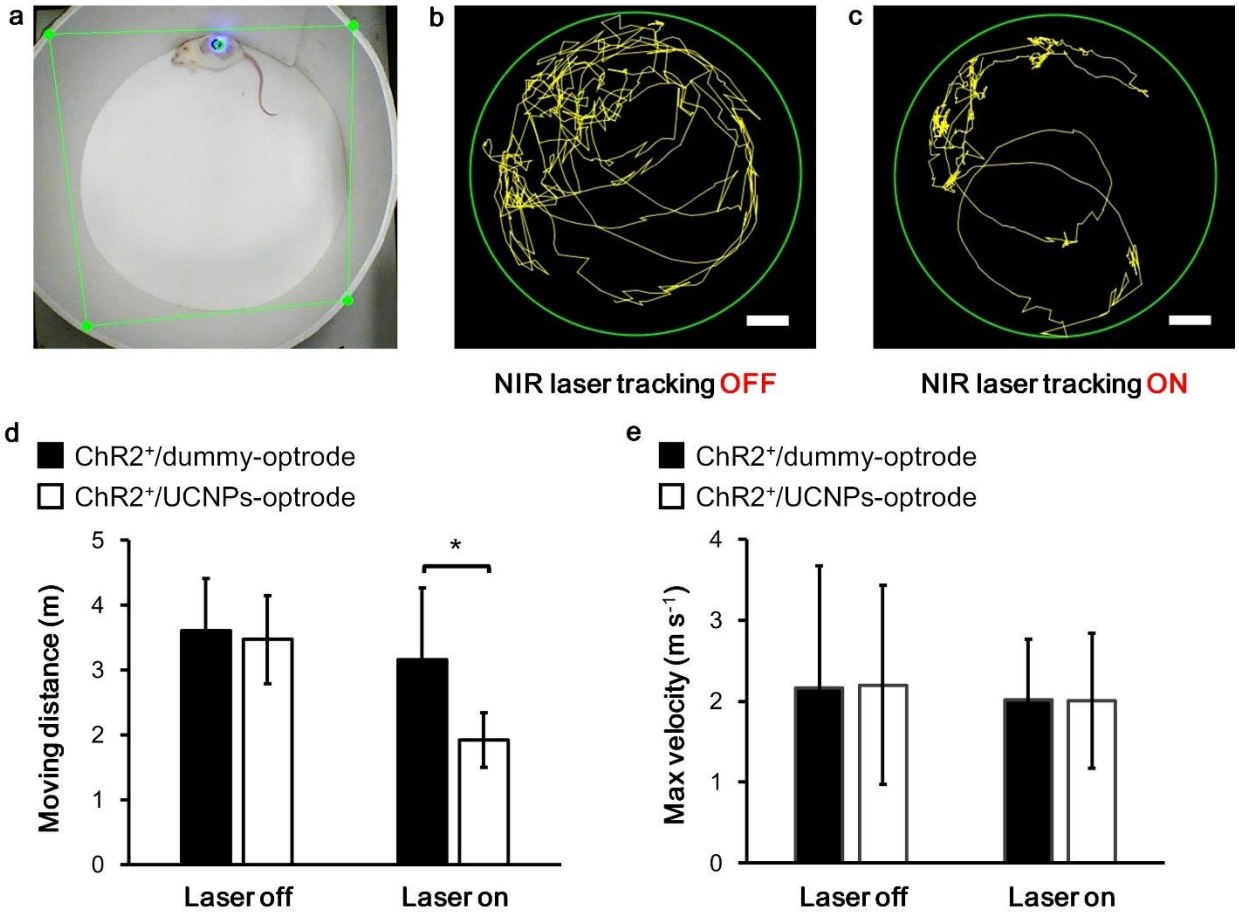

Figure 4. Wireless optogenetics stimulation at the spinal cord of freely-moving mice in the open field test. (a) Mouse was housed inside a holding chamber of the NIR laser projection system. The green circle denotes the laser aiming region of UCNP-PP-optrode. (b-c) The movement trajectory of ChR2-transfected mouse implanted with UCNP-PP-optrode during the first two-minute period when NIR laser stimulation was turned off (b) and during the following two-minute period when NIR laser was turned on (20 mW mm^{-2} power density, 1 Hz frequency, 50 ms pulse width; c). Scale bar, 5 cm. (d-e) Quantitative analysis of moving distance (d) and maximum moving velocity (e) of mice implanted with dummy or functional optrode under the circumstance of tracking NIR laser on and off (n=3). * indicates $P<0.05$ by student t-test. For all panels, the error bars indicate standard error.

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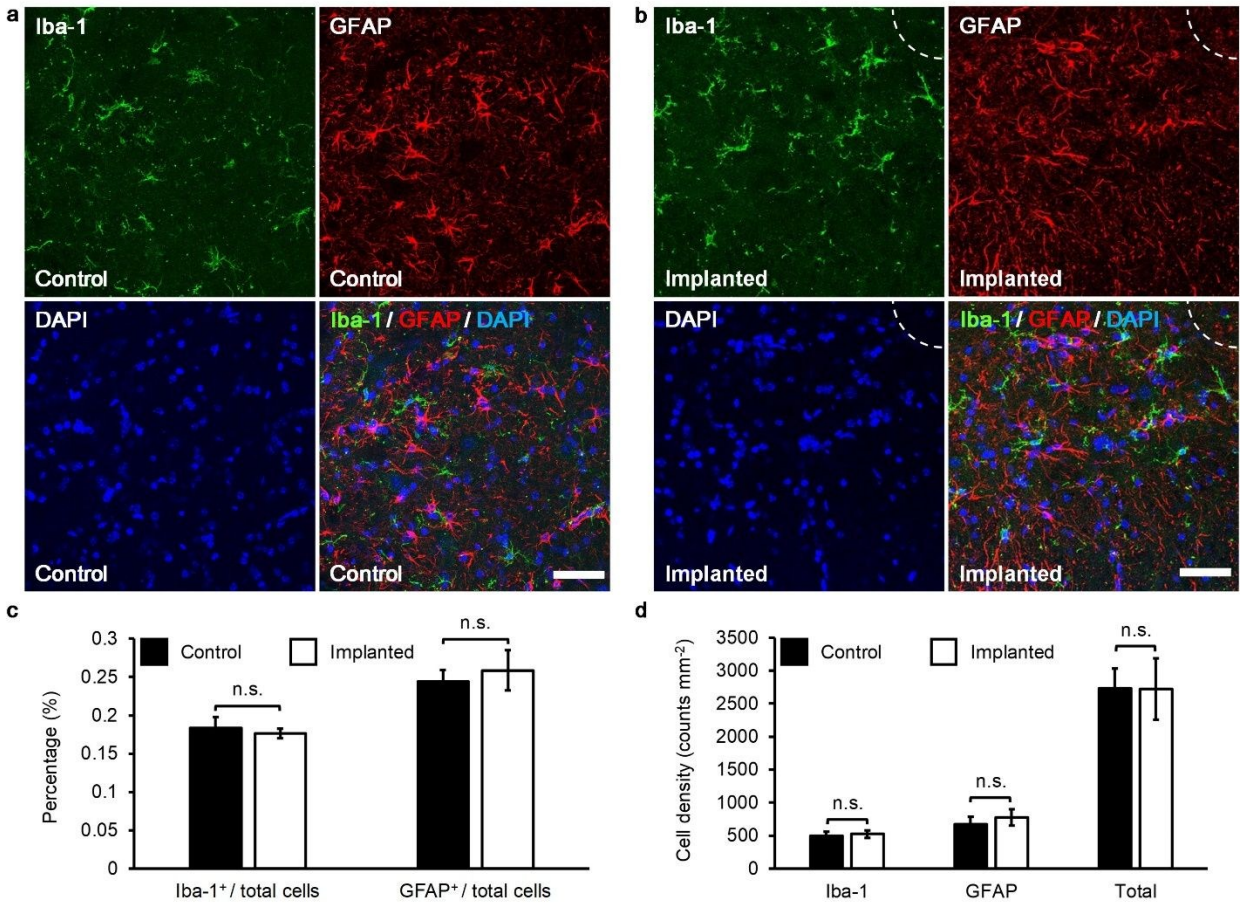

Figure 5. Biocompatibility test for long-term implantation of UCNP-PP-optrode *in vivo*. (a-b) Immunostaining of intact spinal cord tissues of mice (a) and mice with UCNP-PP-optrode implantation for four months (white dashed circle; b). The samples were stained for GFAP (astrocyte marker) and lba-1 (microglia marker). Scale bar, 50 μm. (c) Percentages of microglials and astrocytes in the spinal cord tissues with and without implantation (n=5). (d) Cell density of the spinal cord tissues with and without implantation (n=5). 'n.s.' denotes 'not significant' by student t-test. For all panels, the error bars indicate standard error.

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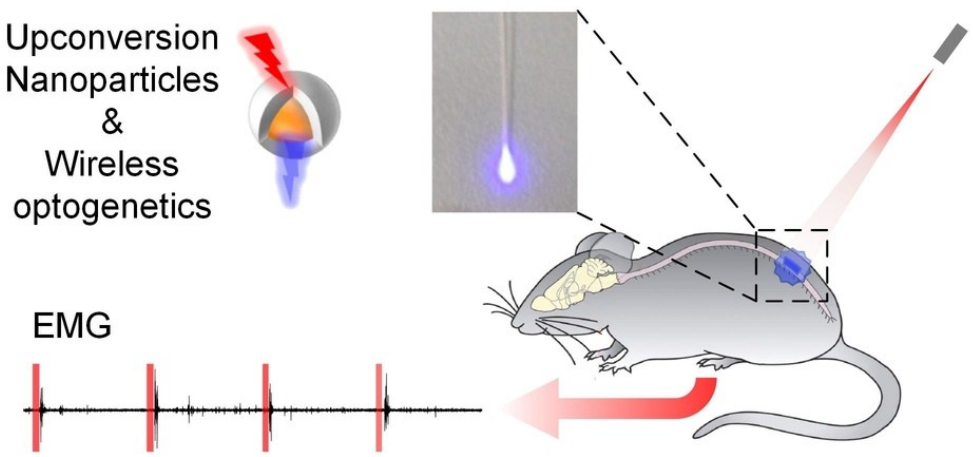

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