Mechano-Neuromodulation of Autonomic Pelvic Nerve for Underactive Bladder: A Triboelectric Neurostimulator integrated with Flexible Neural Clip Interface

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Supporting Information

Contents

Section S1. Detailed configuration and characterization of the stack-layer TENG

The detailed configuration of the 4-layer stack TENG used for the study is shown in Figure S1(a). The contact surface pair is Al(Aluminum)-PTFE(Polytetrafluoroethylene). Devices with different dimensions were prepared: 1×1 cm^2, 2×2 cm^2 and 4×4 cm^2. Since the current is the most essential parameter for nerve stimulations, a detailed current characterization of the short circuit current for the devices with different dimensions and layers was performed, shown in Figure S1(b). As seen, the maximum current for the 4-layer device with 4×4 cm^2 area is about 6 µA. The relationship between the current and the number of layers is not totally linear. This is because the current generated by the different layers cannot be completely synchronized. The four layers have their individual contact at different time points, thus the peaks of their current cannot be overlapped, resulting in an extension of current pulse width. Figure S1(c) shows fabricated TENGs with the difference dimensions.
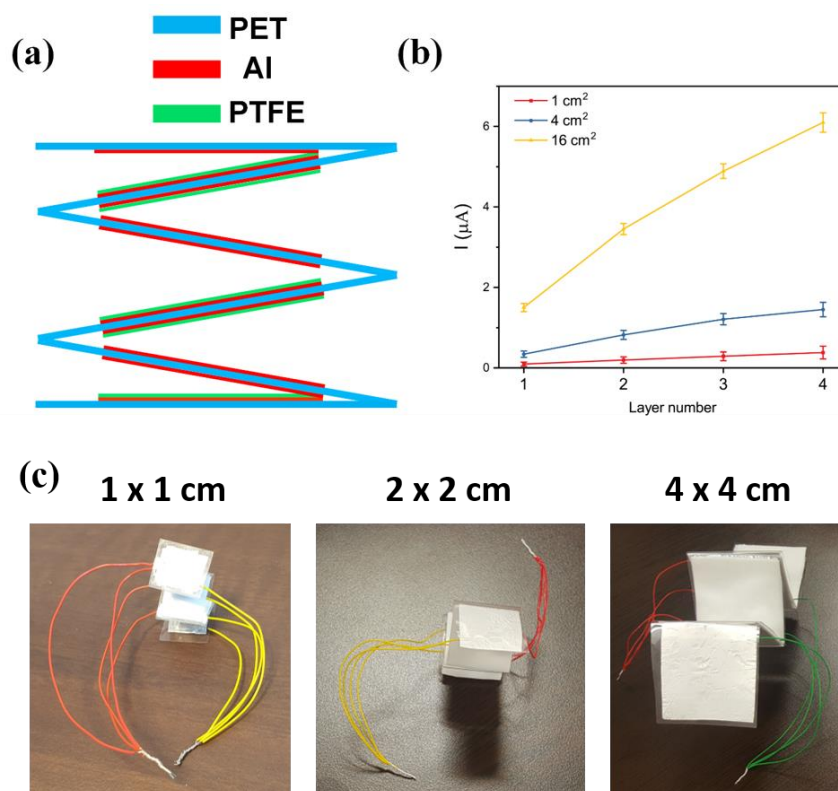

Figure S 1. Detailed configuration and characterization of the stack-layer TENG

Section S2. Preparation of Flexible Neural Clip Interface

The flexible neural clip (FNC) consisted of a polyimide-Au-polyimide sandwiched structure fabricated by micro-electro-mechanical system (MEMS) technology. The fabrication process follows standard photolithographic and clean room procedures. Firstly, a 1 μm thick aluminum (Al) layer was evaporated onto the silicon substrate by physical vapor deposition. It acted as a sacrificial layer to release the final device from the substrate. Then a 8 μm base layer of photosensitive polyimide (Durimide 7505, Fujifilm, Japan) was spun onto the Al coated substrate with a speed of 2000 rpm. After exposure under ultraviolet (UV) light with a dosage of 120 mJ cm^{-2}, the base layer was post-baked and developed in HTRD2 and RER 600 (Fujifilm, Japan), which defined the bottom layer pattern of the FNC. The base polyimide layer was cured at 300 °C in N_2 for 1 hour. In this way, it creates a chemically and physically stable surface for further processing. After that, a layer of AZ 9260 (AZ Electronic Materials, USA) was spun onto the polyimide base layer. This AZ layer was exposed and the electrode traces were patterned. A layer of 20 nm chrome (Cr) was deposited to improve the adhesion of the next conduction layer by sputtering. After a 300 nm gold layer was deposited, the conductive metal layer was patterned by a lift-off process in acetone. Another 8 μm top layer of polyimide was spun onto the processed metal layer, and patterned to expose the sensing contacts and connection pads (Figure S2(a)). Then, we adopted an anodic metal dissolution approach to release the whole device that not only ensured a flat planar structure was released (**Figure. S2(b))**, but also was significantly faster than the traditional wet etching process. Briefly, the wafer was immersed in a 2 M NaCl solution, and connected to an external positive terminal of a voltage source at 1 V. A platinum (Pt) mesh electrode was connected to the negative terminal. A magnetic stir bar was also put inside the solution to keep the concentration of NaCl uniform. After around 20 minutes, the exposed portions of the Al sacrificial layer were removed, and only the covered portions of the Al sacrificial layer were left. Since the contact area between the Al sacrificial layer and the NaCl solution decreased, the current dropped, and the Al etching rate was reduced. Thus, the voltage was then increased to 20 V to speed up the release process. After the entire Al sacrificial layer was removed after 2 hours, the final device was released.

To avoid possible mechanical damages during *in vivo* experiments, reliable and flexible DFT wires (Fort Wayne Metals Research Products Corp.) were connected to the pads of the neural interface using a conductive epoxy (Figure 2(c)). Then, biocompatible parylene C was deposited on the device (except the sensing electrodes) to isolate electrically connected parts from the wet environment inside the body, as well as to reduce foreign body reaction for chronic implantation (Figure S2(d)). Finally, Kwik-sil (World Precision Instrument) was applied to the connection part of the device to provide another layer of encapsulation to prevent liquid leakage, as well as to enhance mechanical support (Figure S3(e)).
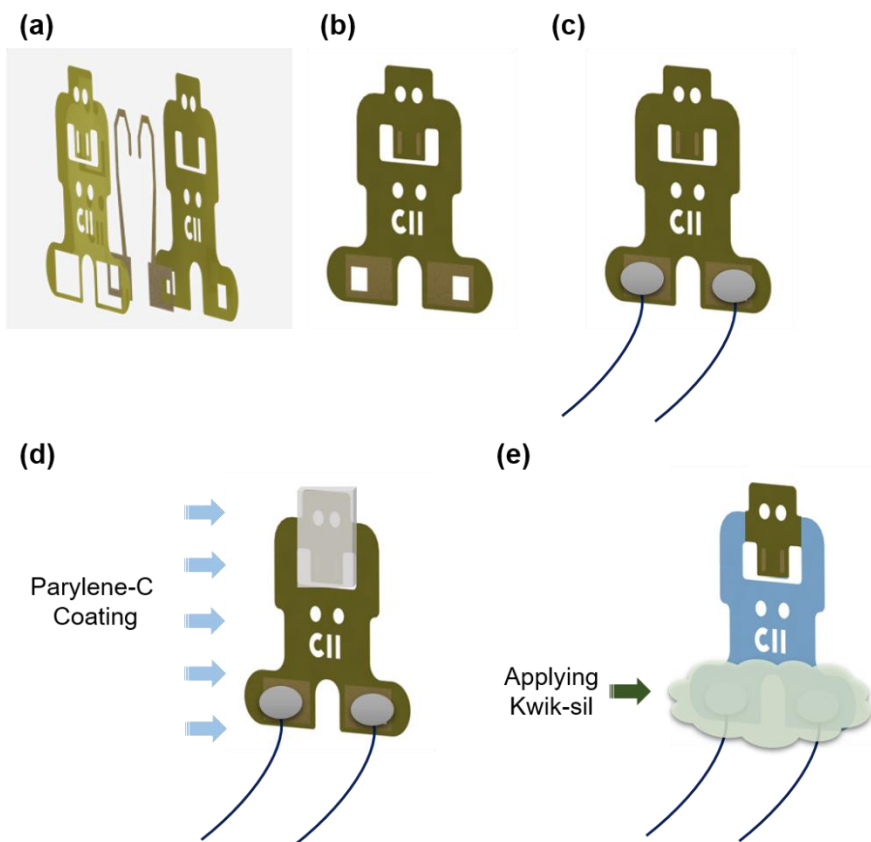

Figure S 2. Fabrication and preparation of flexible neural clip (FNC) interface

Section S3. Characterization of Flexible Neural Clip Interface

Iridium oxide coating

The electroplating solution was used for iridium oxide coating to enhance stimulation performance. 300 mg of iridium chloride was dissolved in 200 ml of DI water, and stirred for 15 minutes. Then, 1000 mg of oxalic acid powder was added to the solution, and stirred for 10 minutes. Potassium carbonate was slowly added to the solution to adjust the pH to 10.5. The prepared solution was kept at room temperature for 2 days. When it turned into a violet color, it was stored in a dark bottle at 4 °C in the fridge. To electroplate the electrode sites with iridium oxide, a three-electrode configuration with a silver/silver chloride (Ag/AgCl) electrode, and mesh Pt electrodes for the reference and counter electrodes, was used for initial coating. A triangular voltage pattern of 0.55 V was applied 50 times by a potentiostat (Zennium E, ZAHNER-elektrik Inc, Germany). Thereafter, the electrode pads were connected to the negative terminal of an external voltage source, and the positive terminal of the external voltage source was connected to a platinum mesh electrode immersed in the solution together. Pulsed voltage, with peak-to-peak magnitude of 0.55 V and offset voltage of 0.275 V at 1 Hz was applied for 20 minutes to plate the iridium oxide.

Electrochemical Characterization

Iridium oxide coating leads to the rough and porous surface that has high electrochemical surface area (ESA) or real surface area. It enhances surface chemical reactions due to a higher Helmholtz capacitance than that of geometric surface area (GSA). This increases charge injection capacity (CIC) by dropping the electrode-electrolyte capacitance voltage, avoiding occurrence of unwanted redox reactions.[1] The output impedance was recorded with the impedance analyzer. The IrO_2 showed a good impedance value (1.9 ±0.09 kΩ at 1 kHz, n=10), and a cathodic charge storage capacity (56.4 ±2.42 mC/cm^2, n=10) for the stimulation.[2-4] These values are comparable to materials used previously in the literature for neural stimulation.

Section S4. Rat preparation for *in vivo* test

Surgical implantation

Adult female Sprague-Dawley rats (200-300g) were used for acute *in vivo* experiments in this study. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the National University of Singapore. The surgery was carried out in accordance with the 143/12 and R15-0592 protocol. For each experiment, the rat was anesthetized with a mixture (0.2 ml 100 g^{-1}) of ketamine (37.5 mg ml^{-1}) and xylazine (5 mg ml^{-1}) intraperitoneally (I.P.), and supplementary doses of 0.1 ml 100 g^{-1} were injected for maintenance. For the bladder experiment, the animal was placed in the supine position, and a ventral midline incision of the lower abdomen was first made to expose the bladder and then extended laterally to expose the pelvic nerve. The underlying muscles were cut, and adipose and connective tissues were removed or pushed aside to expose about 2 mm of the nerve for electrode implantation.

Physiological Characterization of bladder functions

Bladder pressure and urine output were also measured and detected during the stimulation to quantify the functional output. The FNC, which was connected to a stimulator (AM systems 2100 isolated pulse stimulator, the size of 450 x 250 x 100 mm) (**Figure. S3**) via a FPC connector, was positioned over the nerve using micromanipulators, and 'opened' manually using tweezers for the iridium-oxide coated leads to interface with the nerve at either short or long inter-active lead distances. Stimulation parameters were biphasic rectangular waveforms, with a frequency of 10 Hz, 150 μs phase width, duration of 5 seconds, and amplitudes ranging from 25 to 200 μA. Intra-bladder pressure was measured via a saline-filled catheter (Instech Laboratories Inc), inserted into the bladder that was connected to a pressure sensor (Transpac® IV). An infusion pump for refilling

the bladder was used when necessary. A pair of wires (part of a voltage divider circuit) was placed outside the urethral meatus to detect voiding or urine outflow. A data acquisition board (PicoScope® 4424) was used to acquire amplified pressure signals, sync pulses from the stimulator, and voltage changes from the urine detection wires at a sampling frequency of 20 KHz. All acquired data were then analyzed using custom MATLAB programs. Pressure data were low-pass filtered at 30 Hz, and a 5 second period prior to each electrical stimulation was taken as baseline to calculate changes in pressure.

Figure S 3. A photo of a commercial stimulator (AM System 2100 isolated pulse stimulator)

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