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Carbon nanotube (CNT) yarn fabrication for chronic interfacing with the autonomic nervous system

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ABSTRACT

The ability to reliably and safely communicate chronically with small diameter (100–300 µm) autonomic nerves could have a significant impact in fundamental biomedical research and clinical applications. However, this ability has remained elusive with existing neural interface technologies. Here we show a new chronic nerve interface using highly flexible materials with axon-like dimensions. The interface was implemented with carbon nanotube (CNT) yarn electrodes to chronically record neural activity from two separate autonomic nerves: the glossopharyngeal and vagus nerves. The recorded neural signals maintain a high signal-to-noise ratio (>10 dB) in chronic implant models. We further demonstrate the ability to process the neural activity to detect hypoxic and gastric extension events from the glossopharyngeal and vagus nerves, respectively. These results establish a novel, chronic platform neural interfacing technique with the autonomic nervous system and demonstrate the possibility of regulating internal organ function, leading to new bioelectronic therapies and patient health monitoring.

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MATERIALS

Fe/Al-coated Si/SiO_2 wafers
Quartz tube furnace
High-purity (99.99%) acetylene
Ar/H_2 (2.5:1 v/v)

25 cm long (1 × 7 × 25.4 µm: 127 µm diameter) metal-to-metal composite wire with a silver core (28%) and a stainless steel outer tubing coated with PFA insulation (35NLT®-DFT® wire, Fort Wayne Metals)
Conductive epoxy resin (H20E, EPO-TEK)
Parylene-C
6 mm long (0.51 mm ID, 0.94 mm OD) silicone tube (2415500 - Dow Corning Corporation)
Silicone elastomer (MED-4211/MED-4011, NuSil Silicone Technology)
Tungsten microneedle (UEWSGKSNXNND, FHC Inc.)
3 × 4 cm, 25 µm diameter Platinum-Iridium (Pt-Ir) wire
Conductive epoxy

Adult male Sprague Dawley rats (RRID:RGD_70508, Charles River Laboratories) 350–450 grams
2% isoflurane in 1 L/min oxygen
A custom made hook (Fig. 1c, Supplementary Fig. 3a)
Three-axis micromanipulator
A custom made percutaneous silicone plug
Electrical connector (MCP-05-SS, Omnetics Connector Corporation)
UV curable, medical grade epoxy (OG603, Epoxy Technology Inc.)
Biodegradable 3-0 sutures (Vicryl Plus, Ethicon)
4-0 sutures (Webpro™, Patterson Veterinary)

Mating connector (MCS-05-DD, Omnetics Connector Corporation)
Electrical connector (NPD-36-AA-GS, Omnetics Connector Corporation)
16-amplifier, neural instrumentation amplifier board (C3313/RHD2216, Intan Technologies LLC)
Acquisition system (C3100/RHD20000 Evaluation System, Intan Technologies, LLC)
Manual trigger switch

Carbon nanotube (CNT) yarn fabrication

1 VA-MWCNT arrays (with MWCNTs of *ca.*250 µm in length and*ca.*30 nm in diameter) (Fig. 1a), were first synthesized on Fe/Al-coated Si/SiO_2 wafers using a low pressure chemical vapor deposition (CVD) method according to our previously reported procedure[52].

2 A 10-nm thick Al layer was coated on the silicon wafers before the deposition of 3-nm thick Fe film.

3 The catalyst coated substrate was then inserted into a quartz tube furnace for the VA-MWCNT array growth using high-purity (99.99%) acetylene as the carbon source and Ar/H_2(2.5:1 v/v) as a carrier gas under 0.2 atm pressure at 760 °C[52].

4 For spinning VA-MWCNT arrays into the CNT yarn (Fig. 1a), one piece of the VA-MWCNT array of approximately 3-mm width on Si/SiO_2 wafer was initially peeled off from the edge of the sidewall, drawn away from the CNT array, and attached onto a tip of the rod, which was connected to a motor[27].

5 A CNT yarn was spun by the rotating rod that was moved away from the array, and at the same time the bundle was rotated anti-clock-wise to form the CNT yarn (Supplementary Fig. 1b). The rotating speed was in the range of 2000–20000 rpm and the diameter of the as-prepared yarn was in the range of 10–20 µm, which was controlled by the number of CNTs initially pulled out from the array. The yarn diameter was determined by SEM images.

CNT yarn electrode assembly process

6 The implant electrode assembly is comprised of two different wire types: CNT yarn for implantation in the nerve and a more robust lead wire used to route the electrode under the skin to a percutaneous electrical connector.

6.1 The electrode lead wire is a 25 cm long (1 × 7 × 25.4 µm: 127 µm diameter) metal-to-metal composite wire with a silver core (28%) and a stainless steel outer tubing coated with PFA insulation.

6.2 The DFT® wire insulation was stripped by approximately 3 mm at one end and joined to the CNT yarn by conductive epoxy resin.

7 These wire assemblies were then placed and fixed on a custom-made acrylic rack (Supplementary Fig. 2a) for insulating the CNT yarn wire and junction.

8 Parylene-C was chosen to be the insulation material for the CNT yarn. The Parylene-C thickness was 3.5 µm and was deposited using chemical vapor deposition (CVD).

8.1 During the CVD coating process, a 500 µm portion of the CNT yarn end was left uninsulated by using tape to mask the desired area.

8.2 After the Parylene-C insulation was deposited, the individual electrode wire assemblies were removed from the rack.

9 The junction between the CNT yarn and the DFT® lead wire was placed within a 6 mm long (0.51 mm ID, 0.94 mm OD) silicone tube.

10 Any empty space inside the silicone tube was filled with silicone elastomer to further insulate the wire-to-wire junction.

11 The uninsulated CNT yarn end and a short portion of the insulated yarn was wound around the tip of a Tungsten microneedle

12 The silicone tube between the CNT yarn and DFT® wire was fixed to the microneedle using a slip knot made of thread (Supplementary Fig. 2b).

13 After winding, 3 mm of DFT® wire insulation at the free end was stripped to be eventually soldered to a percutaneous electrical connector.

14 A ground electrode (Supplementary Fig. 2c) was made with a 3 × 4 cm, 25 µm diameter Platinum-

Iridium (Pt-Ir) wire and DFT® wire.

15 The Pt-Ir wire and DFT® wire were joined together by conductive epoxy.

16 This ground electrode was placed chronically under the dorsal skin of the animal.

Chronic In vivo implantation procedure

17 All surgical and experimental procedures were done with the approval and oversight of the Case Western Reserve University, Institutional Animal Care and Use Committee to ensure compliance with all federal, state and local animal welfare laws and regulations.

18 Adult male Sprague Dawley rats (350–450 grams, Charles River Laboratories) were used for all peripheral nerve implants discussed.

19 Prior to surgery, all surgical instruments and implant assemblies were autoclave sterilized at 250 °C for 1 hour.

20 During surgery, the animals were gas anesthetized under 2% isoflurane in 1 L/min oxygen.

21 For each of the nerves, a section approximately 3–4 mm was completely separated from and devoid of all connective tissue.

22 A custom made hook (Fig. 1c, Supplementary Fig. 3a) was attached to a three-axis micromanipulator and positioned to provide a small amount of tension and suspend the nerve in the air.

23 The slip knot was then removed, thereby releasing the silicone tube junction from the microneedle.

24 The CNT yarn electrode assembly was then advanced into the nerve until all of the uninsulated CNT yarn segment was within the perineurium.

25 Micro tweezers were then used to apply a small amount of pressure at the implant site while the Tungsten microneedle was carefully removed leaving the CNT yarn inside the nerve (Supplementary Fig. 3b).

26 The hook was then advanced along the nerve to the next implant site, approximately 2 mm away, and the implant process was repeated.

27 The CNT yarns and the silicone tube junctions were then carefully positioned within the incision site and completely encapsulated with 1 mL of fibrin glue (Supplementary Fig. 3c) to secure the implant in place while the normal physiological process of collagen encapsulation took place while the fibrin glue was absorbed by the body.

28 The free DFT® wire ends are then tunneled and externalized at a location on the back between the shoulder blades.

29 The free DFT® wire ends and the ground wire are then passed through a custom made, percutaneous silicone plug and soldered to an electrical connector.

30 The connector was then fitted inside the silicone tube of the percutaneous plug and the tube was filled with UV curable, medical grade epoxy.

31 Blunt dissection is then performed to create a pocket in the back between the skin and muscle.

32 The implanted wires and ground wire are placed within this pocket.

33 The percutaneous plug's mesh base was then sutured to the underlying muscle fascia using biodegradable 3-0 sutures.

34 All skin incision sites were closed using 4-0 sutures.

Chronic autonomic nerve recordings

35 The recording system was designed to accommodate two, differential recording channels. Each recording channel has eight amplifier hardware averaging for increased signal-to-noise recording results[53]. This was achieved by creating, two custom, printed circuit boards (PCB) (Supplementary Fig. 4a and b).

35.1 The first PCB contains the mating connector to the connector which resides in the implanted percutaneous plug.

35.2 The second PCB routes each differential electrode pair to an electrical connector that connects directly to a commercially available 16-amplifier, neural instrumentation amplifier board.

36 DFT® wires are soldered to make electrical connections between the two PCB boards for all the

CNT yarn electrode implants (up to four total) and the implanted ground wire.

37 The electrode connections are routed such that one differential channel is simultaneously recorded with amplifiers 0–7 and the other is captured with amplifiers 8–15.

38 The RHD2216 performs the signal filtering, analog signal multiplexing, and analog to digital conversion and is controlled via a digital SPI connection to a commercially available acquisition system (Fig. 1eand Supplementary Fig. 4c).

39 Neural signal acquisition was performed and stored to a laptop computer using the following system configurations: 20 k samples/sec/amplifier, DSP offset removal was enabled with the high-pass filter cutoff frequency set at 100 Hz, the amplifier bandpass filters were configured to be between 100 Hz and 9 kHz.

40 A manual trigger switch signal is recorded via a separate analog-to-digital input line on the RHD2000 evaluation system.

40.1 This trigger signal is synchronized with the recorded neural amplifier data and is also sampled at a rate of 20 k samples/sec.

40.2 Holding down a simple push button switch, produces a high logic-level trigger signal which is used to mark different events during the various experiments.

40.3 Releasing the push button returns the trigger signal to a low logic-level (Supplementary Fig. 4c).