MRI COMPATIBLE MULTIFUNCTIONAL CARBON NANOFIBER NEURAL PROBE

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ABSTRACT

In this work, a neural probe with carbon nanofiber (CNF) electrode and dual microfluidic channel that fabricated with partial exposure method has been demonstrated and tested. The probe has high resolution electrodes array that can achieve simultaneous electrophysiological modulation and drug delivery or fluid sampling. The probe is MRI compatible since it made from carbon and SU8 epoxy. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) scan have been applied to the probe to show the electrical properties. The structure and property of microfluidic channels are demonstrated by 3D reconstruction of nano computerized tomography (CT) scan and flow rate test with portable micropump.

KEYWORDS

Multifunctional Neural Flexible Probe, Carbon Nanofiber, MRI Compatible, Partial Exposure

INTRODUCTION

Ever since the first neural signal collected by tungsten wire in 1957 [1], neural probes capable of electrophysiological recording have helped neuron science researchers to develop deeper understanding to the dynamics of neuron cells, brain circuits and their relationship with animal behaviors [2]–[5]. After years of evolution, the neural probes nowadays can not only detect or deliver electrical signals, but also capable of drug delivery, fluid sampling and optical signal transmitting for optogenetic applications [6]–[8] These additional functions enable the bi-directional communications and lead to higher spatial or temporal resolutions.

Besides the commonly used electrical modulation, chemical modulation also plays an important role in neuropathology, such as the characterization of dysfunction in distinct brain circuits[9], [10]. Existing technologies like functionalized field-effect transistors, cell based biosensor and microdialysis tools enables the in vivo small and widely distributed neurotransmitter sensing[11], [12]. However, the neuropeptide, protein and other rare larger neurochemicals are left untouched[13]. Micro-invasive tools for brain interstitial fluid (ISF) sampling can assist the characterization of multiple biomarkers[14]. And the combination of the microfluidic channel and microelectrode array can further explore the electrical and chemical signals inside the brain.

Probes with microfluidic channel can also infuse drug cross brain blood barrier and avoid off-target effect of systemic injection. For example, such probes can infuse designer drug exclusively to designer receptors in the targeted brain region and monitor the electrical signals simultaneously [15], [16]. Instead of using steel canula, viral particles with specific gene can be delivered with the probe with fluidic channel to alter the gene expression of certain tissue to make it sensitive to optical stimulation[17]. Probe with microfluidic channel can infuse drugs like dexamethasone to reduce the inflammatory response right after insertion for signal to noise ratio (SNR) improvement [18].

Neural probes provide excellent spatiotemporal resolution at the implantation sites; however, the brain circuits contain multiple levels that highly corelated and work simultaneously. Though implant more probes into brain can increase spatial coverage, it's still difficult to record the entire brain. The magnetic resonance imaging (MRI) has lower temporal resolution but come with higher spatial coverage for entire brain. Also, the functional MRI (fMRI) can monitor the blood oxygen levels in the brain which inform researchers about the active level of brain regions. Thus, the combination of neural probe and MRI can effectively enhance the spatiotemporal resolution for neuron interrogation.

The major problem for combining conventional neural probes with MRI scanning is the artifacts around the implantation site that masks the images from tissue. The artifact is caused by the magnetic susceptibility mismatch between the electrode's materials and brain. Conventional materials for microelectrodes like tungsten, platinum, iridium have larger magnetic susceptibility mismatch with the brain than carbon. Therefore, electrode made from carbon can greatly reduce the MRI artifact size and offer higher spatial resolution. In addition, carbon electrode with higher resistivity has lower chances to cause injury due to eddy current induced heating during MRI compare with metals[19]. Thus, we would like to use carbon as electrode materials for electrophysiological modulation.

Carbon electrodes have different forms including graphene for transparent electrodes array, vertical aligned carbon nanotube electrodes for high surface area, glassy carbon for electrochemical stability, and carbon nanofiber pillar for better neuron cell adhesion [20]–[22]. Among those of carbon structures, carbon nanofiber derived from electrospun photoresist fiber pyrolysis has been chosen as electrodes for facile fabrication with lower cost.

Foreign body response is the primary cause of neural probe implants failure[23], [24]. This is caused by the trauma during surgical implantation and the post-surgery chronic micromotion between mechanically mismatched implant and brain tissue. Probes made from silicon or glass has young's modulus several magnitudes higher than brain, and cause scar tissue formation [6]. Such issue can be mitigated by using materials with lower young's modulus and design with smaller bending stiffness.

In this work, we present a neural probe with carbon nanofiber electrodes and two microfluidic channels for drug delivery and sampling. The carbon electrodes can lower the MRI artifacts around the tissue while CNF structure for cell adhesion promotion[19]. SU8 epoxy was used as mechanical structure for the probe to provide sufficient mechanical strength for surgical implantation while maintaining lower bending stiffness to reduce chronic tissue response.

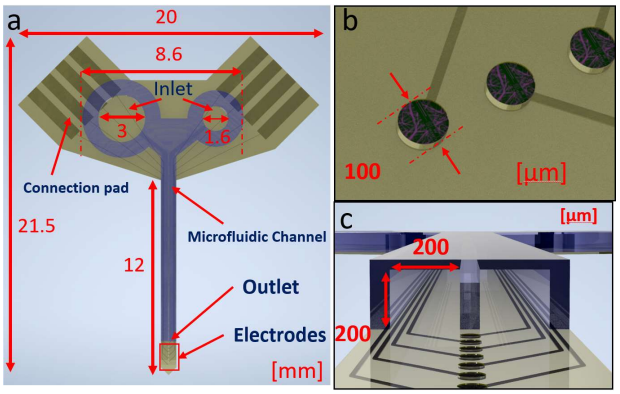

Figure 1. (a) Design details of CNF multifunctional neural probe (b) Carbon nanofiber electrodes (c) microfluidic channel outlet

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NEURAL PROBE STRUCTURE AND DESIGN

The design goal of probe in this work is combining the carbon nanofiber electrodes for electrophysiological modulation and microfluidic channels for liquid sampling or drug delivery. SU8 photosensitive epoxy was chosen as the only materials for the probe design. SU8 as structural material for neural probes can be easily made into different form factors with microfabrication techniques [25], [26]. The microfluidic channel and substrate are made from SU8 for mechanical support and passivation while CNF electrodes and carbon traces are made from SU8 pyrolysis. Design details and dimension of the probe are shown if Fig 1 (a), the 100µm CNF electrodes that interact with neuron tissue are connected to zero insertion force (ZIF) connection pad with 20µm glassy carbon traces. The microfluidic channels have two outlets with different size that designed to accommodate tubes with different dimensions to connect with external pumps for actuation. In Fig 1(c), two microfluidic channels with 200x200 μm^2 cross-section area and opening adjacent to electrodes are placed on top of the substrate

Having sufficient mechanical strength to survive the surgical implantation and lowering bending stiffness to reduce scarring effect during post implantation micromotion are the two primary design concern of polymer neural probes. Previous paper has demonstrated that polymer shaft probe with sufficient buckling force is compatible with conventional implantation process[19] . And according to the literature, the probe need to have at lease 1mN[25] buckling force to survive the surgery. Equation (1) describe the buckling force of probe shank part for insertion in which E, b, h, L are young's modulus, width, thickness, length of the probe shank. Therefore, we design the minimum probe substrate thickness of 40 µm, width of 1mm and probe length of 120mm. The calculated buckling force is 2.98mN for the probe and it's already strong enough to survive the implantation surgery without considering the strengthen from microfluidic channel.

$$F = \frac{\pi^2 E b h^3}{5.88 L^2} \quad (1)$$

After implantation, the micromotion and mechanical mismatch between probe and brain tissue is the primary cause of inflammation and glial effect [7] and lowering the bending stiffness can effectively reduce the it.

$$K = \frac{E b h^3}{4 L^3} \quad (2)$$

According to equation (2), the bending stiffness value K of the same probe design will be 285 Nm^2 for silicon, 120 Nm^2 for glass and 6 Nm^2 for SU8 polymer probe. Comparing with silicon and glass for conventional neural probe, SU8 photosensitive epoxy has lower young's modulus, bending stiffness and thus better for neural probe construction.

FABRICATION METHODS OF NEURAL PROBE

The multifunctional neural probe is entirely made of SU-8 but with different fabrication techniques that including electrospinning, oil immersion exposure, partial exposure, and pyrolysis. The detailed fabrication process is shown in Fig 2. Fabrication starts from silicon wafers with 200nm thermally grown oxidation layers. The A1-A3 steps in Fig 2 show that the SU8 pattern of interconnections with 10um thickness are exposed by standard photolithography process while eight SU8 nanofiber pillars with 100 µm diameter are achieved by electrospinning and consecutive oil immersion lithography[22]. The patterned SU8 nanofiber and traces are turned into CNF and glassy carbon by 3 ºC/min slow ramping pyrolysis at 1000 ºC in 13 slm flowing nitrogen environment (A4 of Fig 2). In A5, SU8 layer with 40 µm thickness is patterned as a substrate of neural probes and in A6, a 250µm thick SU8 layer has been coated on top of it to define the microfluidic channels. The sidewall and top-wall of microfluidic channels whose cross-section area were defined with I-Line UV with 570 mJ/cm^2 fully exposure and 84 mJ/cm^2 partial exposure separately[26]. Then the exposed probe has been baked at 85°C for 15 minutes before 24 hours developing with stirring. The expected thickness with partial exposure techniques is 50µm. After developing, the carbon nanofiber neural probe with two microfluidic channels was released from the silicon substrate by submerging the wafer into buffered oxide etchant (BOE) for 48 hours.

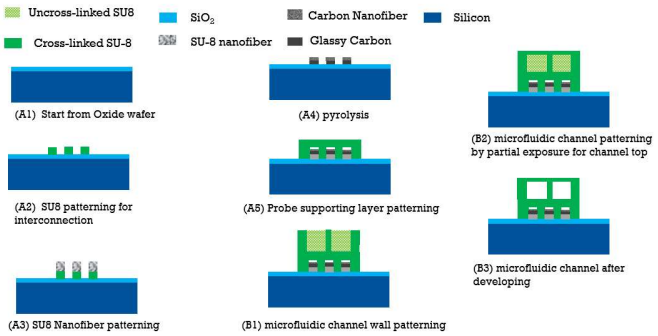

Figure 2. Fabrication process of the multifunctional neural probe, A1-A5 shows the fabrication of carbon nanofiber electrodes and B1-B3 shows microfluidic channel fabrication. (The white space on top of carbon nanofiber electrodes in A5-B3 indicate the opening on shank to expose electrode)

RESULTS AND DISCUSSION

The dimension of electrodes has been examined with scanning electron microscope (SEM) and tested with electrical impedance spectroscopy while the dimensions of microfluidic channels are measured by nano computerized topography (Nano-CT) and tested with micropump system.

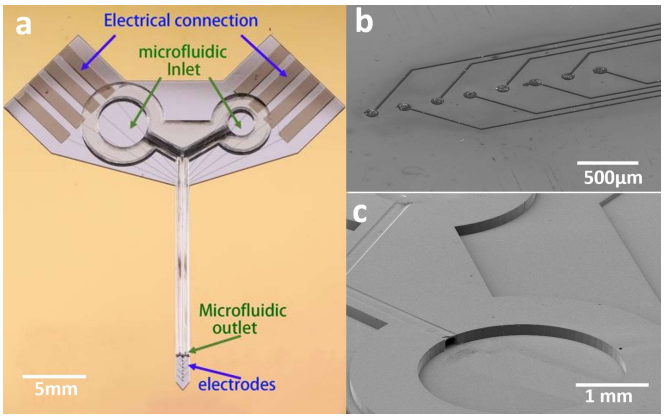

Figure 3. (a) Optical image of the multifunctional CNF neural probe (b) with an SEM image for electrodes and traces (c) microfluidic connection and inlet

Fig. 3 (a) shows the optical image of the fabricated neural probe structure including the inlet and outlet of microfluidic channels and electrical connection to interface with external circuits. The SEM image of carbon nanofiber electrodes and inlet of microfluidic

channel are included in of Fig 3 (b) and (c). Details of the 100 µm diameter carbon nanofibers electrode is demonstrated with SEM imaging in Fig 4. The CNF mesh with thickness around 15 µm sits on top of the glassy carbon trace and the diameter of single carbon nanofiber ranges from 100 nm to 600nm. The 3D mesh structure of the nanofiber provides higher neuron density compare with smooth silicon surface[19].

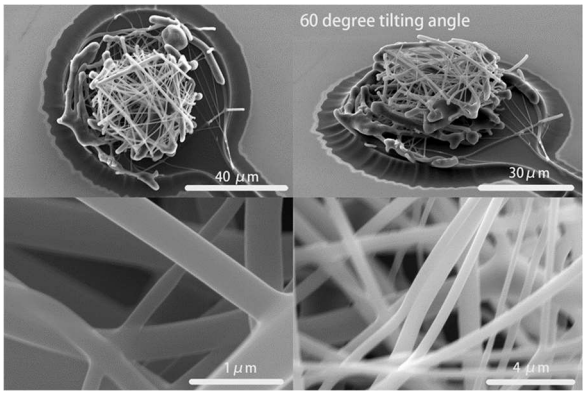

Figure 4. SEM images of carbon nanofiber electrodes

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) are carried out with three-electrode system which include a platinum counter electrode and an Ag/AgCl reference electrode in phosphate buffer saline (PBS) solution. EIS plot in Fig 5(a) shows that the impedance of carbon nanofiber electrodes is around 419±40 kΩ at 1 kHz. The CV curve in Fig 5(b) was measured within water window -0.6-0.8V in. The 10 scan cycles demonstrate the stability of the electrodes, and the approximate rectangular shape is expected for double-layer capacitance electrodes [6].

Nondestructive Nano-CT scanning has been performed to examine the microfluidic channel dimensions. During the scanning, tungsten target has been used to generate X-ray with 180kV 120µA electron beam and 1000 frames scanning have been collected and reconstructed into 3D file. The 3D structures of fabricated probe are cut into slices and shown in Fig 6 (a) and (b). Fig 6 (a) provides the side view and cross-section views of the channels and connection parts. The top wall is much thinner than substrate and sidewall of the probe. Also, top wall formed with partial exposure is continuous across the channel and free of defects that would cause the channel leakage.

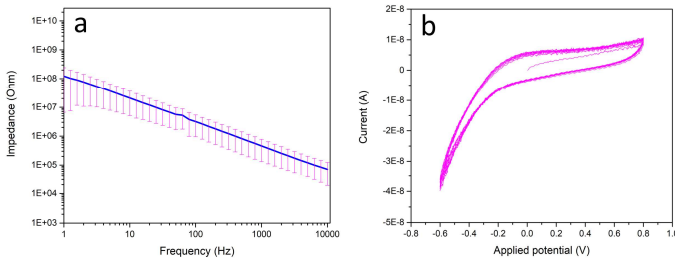

Figure 5. electrical measurement of the CNF electrodes (a) Electrochemical impedance spectroscopy measurement (b) cyclic voltammetry measurement

Fig 6 (b) shows the structure and cross-section view of probe shank whose area is approximately 200x200 µm^2 and top wall thickness is around 50 µm as designed. However, the shape of channel cross-sections is not perfect square but trapezoid and slight changes across slice 1-3. It's formed because of two factors, i) the nonhomogeneous exposure and ii) attenuation effect of SU8 photo resist during the photolithography process. For factor i), the top portion of the side wall absorbed more dosage than the lower part because of partial exposure only provides limited energy that cannot uniformly cover the entire sidewall. For reason ii), the UV light attenuated when passing through the 250 µm SU8 layers, so the lower portion of the sidewall has less crosslinking than top part.

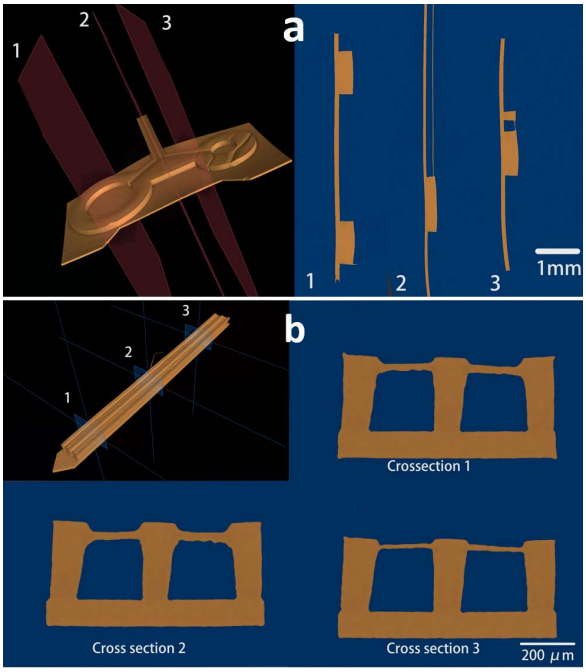

Figure 6. Nano-CT reconstruction view of microfluidic channel (a)connector region (b) shank regions

The microfluidic channel inlet was then connected to a micropump (mp6-liq, Bartels Mikrotechnik) driven by voltage upconverting transformer circuits. Water was used in the test and volume was converted from weight measurement. The pump generates pulsatile flow whose actuation frequency, amplitude can be tuned with external input. In this case we used function generator and source meter to adjust the frequency and amplitude. We chose 0.37V fixed input amplitude and alter pumping frequency for the microfluidic channel test. As shown in Fig. 7, the flow rate increases as frequency getting higher, and the lowest flow rate we achieved here is 10µl/min. Though the continuous flow rate is still higher than normal infusion rate[8], we can control the on/off time as time modulation to achieve lower flow rate.

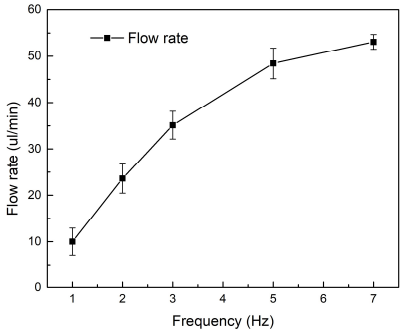

Figure 7. flowrate measurement of microfluidic channel with varying pump frequency

CONCLUSION

The polymer based CNF multifunctional neural probe capable of electrical recording and stimulation, drug delivery and liquid sampling has been designed and fabricated. The probe constructed with SU8 epoxy has suffient mechanical strength to withstand the surgical implantation while the tip has lower bending stiffness to reduce the tissue response over chronic micromotion. The fabrication flow doesn't involve mannual assembly so it has potential for batch manufacturing with lower cost. CV and EIS test has been carried out for the probe and resultant electrical impedance is 420 ±40 kΩ at 1kHz for the CNF electrodes. The CV curve indicates the stability and double layer capacitance mechanism of the elecrodes. The two microfluidic channel fabricated with partial exposure methods has been examined with nano CT scan and 3D reconstruction. They indicate the formation of the 200x200μm microfluidic channels and 50 μm channel top wall. The flowrate of the channels was tested with micropump actuated at different frequencies. The flow rate ranges from 10μl/min to 55μl/min in continous flow and can reach lower flowrate with time modulation. Though the results show validate the fabrication methods and functions of the probe, further in vivo studies can push the probe further as a platform for neural research.

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