Published in final edited form as:

Analyst. 2016 September 21; 141(18): 5256–5260. doi:10.1039/c6an01469k.

Novel Carbon-Fiber Microelectrode Batch Fabrication using a 3D-Printed Mold and Polyimide Resin

Elefterios Trikantopoulos[#], Cheng Yang[#], Mallikarjunarao Ganesana, Ying Wang, and B. Jill Venton^{*}

Department of Chemistry, University of Virginia, Charlottesville, VA 22904

[#] These authors contributed equally to this work.

Abstract

Glass insulated carbon-fiber microelectrodes (CFMEs) are standard tools for the measurement of neurotransmitters. However, electrodes are fabricated individually and the glass can shatter, limiting application in higher order mammals. Here, we developed a novel microelectrode batch fabrication method using a 3D-printed mold and polyimide resin insulating agent. The 3D-printed mold is low cost, customizable to change the electrode shape, and allows 40 electrodes to be made simultaneously. The polyimide resin is biocompatible, quick to cure, and does not adhere to the plastic mold. The electrodes were tested for the response to dopamine with fast-scan cyclic voltammetry both *in vitro* and *in vivo* and performed similarly to traditional glass-insulated electrodes, but with lower background currents. Thus, polyimide-insulated electrodes can be mass-produced using a 3D-printed mold and are an attractive alternative for making cheap, biocompatible microelectrodes.

Introduction

Carbon-fiber microelectrodes (CFMEs) have served as the standard tool for detection of electroactive neurotransmitters *in vivo*.¹ CFMEs have multiple advantages including biocompatibility, fast electron transfer kinetics for neurotransmitters, and good adsorption properties for biogenic amines.^{2,3} The traditional method for CFME fabrication involves threading an individual, cylindrical carbon fiber into a borosilicate glass capillary that is drawn to a sharp tip using a vertical capillary puller.⁴ However, the glass insulation method has several disadvantages.^{5,6} First, glass-insulated CFMEs are fabricated one at a time and require a series of non-automated steps, making mass production difficult. Second, glass electrodes can potentially shatter in tissue, and so they are not permitted for use in higher order mammals.⁷ Therefore, new methods are desirable that reproducibly produce non-glass insulated CFMEs economically on a large scale.

An easy method to mass fabricate small objects reproducibly is to use a mold. Poly(dimethylsiloxane) (PDMS) molds have been made extensively using photolithography but the process requires a master to be made prior to mold production.⁸ Laser etching of

^{*}Corresponding author: jventon@virginia.edu, Phone 434-243-2132.

^{*}Electronic supplementary information (ESI) available: Chemicals and details regarding glass-insulated electrode fabrication

materials, such as Teflon, has been used to design molds for microelectrode fabrication.⁵ However, photolithography and laser etching are restricted by a limited depth of focus and aspect ratio,⁹ and these methods require high cost instruments or long production times. 3D printing is an emerging technology that allows for rapid prototyping with high reproducibility and low cost.¹⁰ The method has the advantage that it can create almost any complex shape or geometric feature; 3D printers have reached extremely high resolution within microns and are constantly being improved.^{11,12} Thus, 3D printing provides an alternative method of mass-fabricating electrochemical devices, such as stainless steel electrodes,¹³ or electrochemical chips for direct biological measurements.¹⁴ However, the fabrication of electrochemical probes with micron diameters has not been reported with 3D printed devices.

Making electrodes in a mold requires alternative insulations other than glass, which may be beneficial for designing electrodes that are biocompatible and shatter resistant. Epoxy resin has been used previously to completely insulate carbon fiber,⁸ and paraffin wax to seal glass capillary CFMEs.⁹ Polyimide-coated, fused silica capillaries have been developed as another method of insulating carbon-fibers for chronic tissue implantation.¹⁵ While these polyimide coated capillaries are sold as a commercial product, polyimide is a mechanically flexible insulator that can be poured into molds and manipulated into unique three-dimensional designs.¹⁶⁻¹⁸ Thus, polyimide has been used as a biocompatible insulator to provide an optimal implant environment and extend the longevity of the tissue-electrode interface.¹⁶

In this work, we develop a 3D-printed mold for *in vivo* microelectrodes and use it to make polyimide-insulated CFMEs. The 3D-printed mold enables a rapid and low cost fabrication of the desired electrode geometry with high resolution features. These electrodes have a similar performance for dopamine detection to traditional glass-insulated electrodes, both *in vitro* and *in vivo*, and can be batch fabricated and customized to different shapes.

Experimental

3D printed mold fabrication

Molds were designed in Autodesk Inventor Professional 2014 Student Edition, converted to an .STL file, and subsequently printed by the Department of Mechanical Engineering at University of Virginia. The 3D printed polymer molds were manufactured on a Stratasys Connex 500 Model 1 Poly-Jet 3D printer (Stratasys Ltd., MN), which has 8 print heads with 96 nozzles per head. Water jets were used to remove the support. Rigid opaque black material (VeroBlackPlus RGD875, mainly acrylonitrile butadiene styrene, Stratasys) was used because of its suitability for rapid tooling with dimensional stability and fine detail. The heads heat up to 60 °C, and the Z axis resolution with this material was 30 µm. Electrodes were fabricated with carbon fibers (7 µm in diameter, T650, Cytec Engineering Materials, West Patterson, NJ). Polyimide sealing resin (Grace Davison Discovery Sciences, Deerfield, IL) was used to fill the mold and provide insulation.

Electrochemical instrumentation

Fast scan cyclic voltammetry (FSCV) was performed using a ChemClamp potentiostat (Dagan, Minneapolis, MN). The waveform was generated and the data was collected using a High Definition Cyclic Voltammetry (HDCV) breakout box, HDCV analysis software program (UNC Chemistry Department, Electronics Design Facility) and PCIe-6363 computer interface cards (National Instruments, Austin, TX). Electrodes were backfilled with 1M KCl and a silver wire was inserted to connect the electrode with the potentiostat headstage. A triangle waveform was applied to the electrode from a holding potential of -0.4 V to 1.3 V and back at a scan rate of 400 V/s and a frequency of 10 Hz. A silver–silver chloride wire was used as the reference electrode. Samples were tested using a flow injection analysis system as previously described.² Buffer and samples were pumped through the flow cell at 2 mL/min using a syringe pump (Harvard Apparatus, Holliston, MA).

Animals

Male Sprague-Dawley rats (250–350 g) purchased from Charles River were housed in a vivarium and given food and water *ad libitum*. All experiments were approved by the Animal Care and Use Committee of the University of Virginia. The rat was anesthetized with urethane (1.5 mg/kg i.p.), the scalp shaved, and 0.25 mL bupivacaine (0.25% solution) given subcutaneously. The working electrode was implanted in the caudate putamen (in mm from bregma: AP + 1.2 , ML + 2.0 , and DV -4.5 to 5.0), the stimulating electrode in the substantia nigra (AP -5.4 , ML + 1.2 , and DV -7.5), and the Ag/AgCl reference electrode in the contralateral side of the brain. The DV placement of the stimulating electrode was adjusted downward until a robust dopamine signal was measured. The polyimide-insulated carbon-fiber electrode was inserted into the brain and the FSCV waveform applied for 30 min to allow the electrode to stabilize. Stimulated release was electrically evoked using biphasic stimulation pulses (300 μ A, 30 – 120 pulses, 60 Hz).

Results & Discussion

Polyimide-Insulated Microelectrode Fabrication Using 3D-Printed Mold

Polyimide-insulated microelectrodes were made using a 3D-printed mold. Figure 1A shows an example of a single channel in the mold. Our design used channels with sharp tips that are 150 μ m in diameter and 3 mm in length that connects to a tapered section that is 5 mm long, making the total polyimide section 8 mm. This length is longer than the conventional penetration depth (4.5 – 5 mm) for the caudate putamen, but the length could be customized for other brain regions or applications.^{19,20} There is also a 15 mm section designed for microelectrode support, which is a cannula needle in this design. Cannula needles made of stainless steel are widely used for neuroscience studies and are safer for brain insertion compared to glass capillaries.²¹ A 23 gauge cannula needle was used as the connector, with inner diameter of 0.34 mm and outer diameter of 0.64 mm, which is half the outer diameter of conventionally used glass capillaries (1.2 mm). Figure 1B shows an image of an actual channel while Fig. 1C shows an electrode after fabrication. The whole mold design includes 40 electrodes per side, and the number of channels and the dimensions can be easily customized by changing the computer-aided design (CAD) parameters.

The process of making the polyimide-insulated CFME is shown in figure 1D. An individual carbon fiber was inserted through a 23 gauge cannula needle, which was laid into the wide part of the mold. Polyimide resin was then poured into the mold channels, filling the tapered and tip part and sealing it to the cannula. It takes about 20 minutes to fill 40 channels with polyimide resin, and the resin was cured for 30 minutes in the oven at 150 °C. An additional layer of polyimide resin can be applied if required and cured for another 30 minutes at 150 °C. The protruding carbon fiber is trimmed in order to make a cylindrical working electrode, typically around 100-150 µm long.

The 3D-printed mold is primarily made of acrylonitrile butadiene styrene (ABS), which provides a non-stick surface for the polyimide resin. The use of this material allowed the removal of the polyimide-insulated CFMEs by simply bending the flexible polymer mold and removing the electrode with tweezers. The 3D-printed molds were reused four times in this study before the detail of the device began to deteriorate. The average cost of materials for each electrode is less than 20 cents, with costs for a batch including only \$1 for the polyimide resin, less than \$0.25 for the carbon fiber, and \$18 for the 3D-printed mold of 40 channels that is used 4 times. The total time-cost for the 40 microelectrode fabrication is less than 2 hours, making for an economical fabrication procedure that can be developed as cheap alternative to the commercially available neurochemical microsensors in the market. For true batch fabrication in the future, filling of the mold could be automated by a robot and the length of the fiber protruding controlled upon laying it in the channel.

The 3D printing technology allowed consistency in electrode production as well as flexibility to change designs if needed. Mold designs can easily be shared as CAD files and then either modified or printed in another location. Thus, this method enables customization in design of the microelectrode system based on the different application requirements. Moreover, our 3D mold fabrication process could be used with other insulating agents such as epoxy⁵ or paraffin.⁶ This fabrication method is not limited to carbon fibers but could be used with many other electrode materials, such as carbon nanotube yarns, or metal wires.^{22,23} 3D printing is an emerging technology that is continuously progressing.¹³ The resolution of the molds varies by printer specifications, and we could make channels as small as 50 µm diameter, but the printing was less consistent and some molds were not well tapered. Thus, we chose channels with 150 µm diameter tips because our printer could reliably and consistently produce these. However, as the method advances, resolution is improved, and high resolution 3D printers becoming more accessible, the fabrication of smaller electrodes with smaller tip diameters will be possible.

Electrochemical Characterization in Vitro and in Vivo

Polyimide-insulated CFMEs were electrochemically characterized for the detection of dopamine using fast-scan cyclic voltammetry (FSCV). Dopamine is an important neurotransmitter in mammalian physiology that regulates locomotion and reward.²⁴ In Figure 2, electrochemical properties of polyimide-insulated electrodes and traditional glass CFMEs are compared. The background-subtracted cyclic voltammograms (CV) of 1 µM dopamine are similar because the length of the protruding fiber was the same, 150 µm. The oxidation peak for dopamine is not statistically different for glass-insulated CFMEs (66 ± 3

nA, n=4) and polyimide-insulated CFMEs (64 ± 2 nA; n=4; t-test, p=0.64); thus, exposure to the polymer 3D printed mold or presence of the insulating polyimide resin does not disturb the reaction of dopamine at the carbon surface. However, the polyimide-insulated CFMEs have smaller background currents (Figure 2B, 1100 ± 100 nA; n=4) than glass-insulated CFMEs (1570 ± 50 nA; n=4; t-test, p=0.0063). The larger background current of glass-insulated electrodes is due to the additional capacitance of the glass, which has a dielectric constant of 6 compared to the dielectric of polyimide, which is 3.²⁵ The thickness of the insulations are also different, with thinner glass than the polyimide, which could allow ions to conduct through the glass. Despite the differences in background charging currents, the dopamine oxidation current of the electrode is not dependent on the type of insulation, as expected.

Figure 2C shows the temporal response of the CFMEs to a bolus of dopamine. The temporal response is not dependent on the type of insulation, although the response is not perfectly square due to adsorption/desorption kinetics.²⁶ The consistent time response proves the polyimide-insulated microelectrodes are well insulated and that the polyimide is not rough at the end, trapping dopamine near the electrode surface.

To evaluate and confirm the electrode stability, the waveform was continuously applied to the electrode while the electrode was immersed in a buffer solution and the response to dopamine characterized over a 4 hour period, a typical time length of an in vivo stimulated release experiment.²⁰ Figure 2D shows the dopamine oxidation signal is constant for 4 hours of continuous scanning and that the surface is stable over the time length of a biological experiment. Shelf life stability was examined by testing electrodes over a month after manufacturing. No change in oxidation current or temporal resolution was observed throughout the one month period (Fig. 2E). The polyimide-insulated CFMEs are stable for several hours of experimental use and have a good shelf life, allowing them to be made in batches and stored until needed.

To determine the applicability of the polyimide-insulated CFMEs as *in vivo* sensors, stimulated dopamine release was measured in anesthetized rats. Stimulation pulse trains were applied (300 μ A, 30-120 pulses, 60 Hz) to the dopamine cell bodies, and the dopamine response was recorded by polyimide-insulated CFME in the caudate putamen. Figure 3A shows dopamine concentrations recorded at different stimulated pulses by polyimide-insulated CFMEs (n=4 rats). Data was converted from peak currents to concentration based on postcalibration factors. As observed in the figure, a higher number of pulses elicit larger release of dopamine on the microelectrode surface, and the electrode is sensitive enough to detect dopamine release as low as 170 nM. Figures 3B and C show example CV and current versus time plots of dopamine detection at a polyimide-insulated CFME *in vivo*.

Polyimide-insulated CFMEs could potentially be used in higher order mammals or humans in the future, given their biocompatibility and shatter resistance. Measurement of dopamine release has been demonstrated previously in the caudate of a human patient during electrode implantation surgery.⁷ The CFMEs used were insulated with polyimide-coated, fused silica capillaries, which are similar in material to the polyimide resin used in this work. The

promising properties of this sensor, as well as the ability to batch fabricate reproducibly, could lead to promising new electrode fabrication methods in the future.

Conclusions

In summary, we have devised a novel method of mass-fabricating CFMEs for the electrochemical detection of neurotransmitters such as dopamine. The polyimide-insulated electrodes are biocompatible and flexible and can be implanted *in vivo*. 3D printing provides an easy way to reproducibly fabricate molds, and electrodes can be customized according to experimental needs. As printing technology continues to evolve, new applications will be enabled, including smaller probes. The polyimide-insulated CFMEs have comparable oxidation currents, stability, and temporal resolution to the conventionally used glass-insulated CFMEs. The electrodes were successfully used as *in vivo* sensors for sensitive detection of stimulated dopamine in caudate putamen. With advantages such as low-cost, high bio-compatibility, easy batch fabrication, and customizable design, this novel microelectrode fabrication method will be helpful for low cost, batch fabrication of carbon and metal material-based microelectrodes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH grants R21DA037584 and R01NS076875 to BJV.

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Figure 1. 3D-printed mold design and polyimide-insulated carbon fiber microelectrodes fabrication process. (A) Close-up of the design of one channel for 3D-printed microelectrode fabrication (B) image of the tip from a 3D-printed mold, scale bar: 2 mm, (C) image of a polyimide-insulated carbon fiber microelectrode fabricated using the mold shown in B, scale bar: 0.5 mm, (D) scheme of the process of microelectrode fabrication.

Figure 2.

Electrochemical characterization of polyimide-insulated CFMEs. (A) Example background subtracted cyclic voltammogram (CV) of 1 μ M dopamine at a polyimide-insulated CFME (black) and glass-insulated CFME (red). (B) Background charging currents for the same electrodes in PBS buffer. (C) Current vs. time plot for the same electrodes during a flow injection analysis experiment; the first arrow indicates the injection of dopamine bolus, and the second arrow shows when flow is switched back to PBS buffer. (D) 4-hour stability test of response to 1 μ M dopamine at polyimide-insulated CFMEs (n = 6). Waveform was continually applied and measurements taken every hour. (E) 30-day long term stability test at polyimide-insulated CFMEs (n = 4), Measurements were taken every 5 days. Data obtained in (D) and (E) were normalized to initial current values for peak oxidation of dopamine.

Figure 3.

Dopamine detection *in vivo* at polyimide-insulated CFMEs. (A) Peak cyclic currents were recorded at different stimulated pulses. Data were converted to concentration on the basis of post calibration factors ($n=4$ rats). (B) Sample CV of stimulated dopamine release detected from microelectrodes placed in the caudate putamen with stimulation pulse trains of 60 pulses at 60 Hz. (C) Associated current vs time plot. Red bar indicates the stimulation duration.