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ARTICLE

Polymer insulation of ultramicro carbon fiber electrodes for electrophysiological, electrochemical and biosensor applications

Dénes Budai[1,2]*, Klára Horváth[1], András Szabó[1]

[1]Kation Europe Bt., Szeged, Hungary, [2]Szeged Neurobiological Knowledge Center, Szeged, Hungary

ABSTRACT There is an obvious need for electrodes with extremely small electroactive areas and structural dimensions that offer great promise for electrochemical microscopy used for neuronal analysis in ultrasmall environments. In the present study we have developed ultramicro carbon fiber (CF) electrodes with combined thin layers of poly(oxyphenylene) and epoxy resin coatings for electrical insulation. The thickness of the borosilicate glass housing and insulating layer of our standard CF microelectrodes is about 1 μm and the carbon tip protrudes by about 20 μm from the glass assembly. Functionalization requires longer sections of the exposed, uninsulated carbon fiber where submicron tips are shaped and chemical modifications are made. Electrodeposition of poly(oxyphenylene) was carried out using anodic currents at 2V against an Ag/AgCl half-cell. After finishing the polymerization at 150°C for 2 hours, electrical impedances of the electrodes were 17.1 ± 2.8 MΩ (mean ± SD, n= 22). An additional epoxy coating was formed by dipping the CF electrodes in diluted epoxy resins followed by dipping in a mixture of diluted curing agents. The epoxy layer significantly increased the effectiveness of the insulation as the impedance for each of the 38 prepared CF electrodes was higher than the upper range limit (200 MΩ) of our impedance meter. The thickness of the combined insulating layer was less than 1 μm as estimated by electron microscopic studies. Removal of the insulation from the very tip was carried out using high voltage spark or electrochemical etching. These submicron CF electrodes are suitable for extracellular spike recording, electrochemical and biosensor applications.
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KEY WORDS

carbon tip etching
scanning electron microscopy
poly(oxyphenylene)
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electrodeposition
electrical insulation
electroactive area

Carbon fiber (CF) microelectrodes are used to record neuronal action potentials (Armstrong-James and Millar 1979; Armstrong-James et al. 1980) or to detect electrochemical signals produced by electroactive compounds such as catecolamines or nitric oxide. The carbon fibers are graphite monofilaments of about 7 μm in diameter. In microelectrodes, they have very high tensile strength, low electronic impedance and they provide oustanding extracellular recording qualities similar to those of the best tungsten electrodes (Budai and Molnár 2001; Budai 2004). By covalent modifications of their surface (Baker et al. 2005; for review see, Downard 2000), CFs are suitable for construction of biosensors on the micrometer scale (Yang 2005; Ahuja et al. 2007; Koncki 2007). In cases of these applications, the base electrode material must be electrically insulated except for a varying section of the recording or sensing tip. CF microelectrodes are usually insulated with borosilicate glass or, less frequently, applying plastic sheating or electrodeposited polymers (El-Deen et al. 2006).

There is an obvious need for electrodes with extremely small electroactive areas and structural dimensions that offer great promise for electrochemical microscopy used for neuronal analyis in ultrasmall environments (*e.g.* single neurons or perhaps even single synapses). This requires an ultrasmall support material and an ultrathin electrical insulating layer that covers the support material except for the very tip of the electrode. In an ideal case, the insulating layer is very thin (less than 1 μm), covalently bound to the carbon surface, it has zero electrical conductivity and the exposed (uninsulated) length of the very tip can be reliably controlled. In the present study we have developed ultramicro CF electrodes with electrodeposited poly(oxyphenylene) insulating layer that are suitable for extracellular spike recording, electrochemical or biosensor applications. The electrical insulation of the submicron CF electrodes was further improved by forming additional layer of epoxy resin.

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*Corresponding author. E-mail: kations@aol.com

Materials and Methods

Manufacturing CF microelectrodes

Single-barrel CF microelectrodes were made from borosilicate glass capillary tubing (1.50 mm o.d., 0.84 mm i.d., WPI, Sarasota, FL). A 15 cm long individual carbon fiber (PAN-based, T-300, Amoco Performance Products, Chichago, IL)

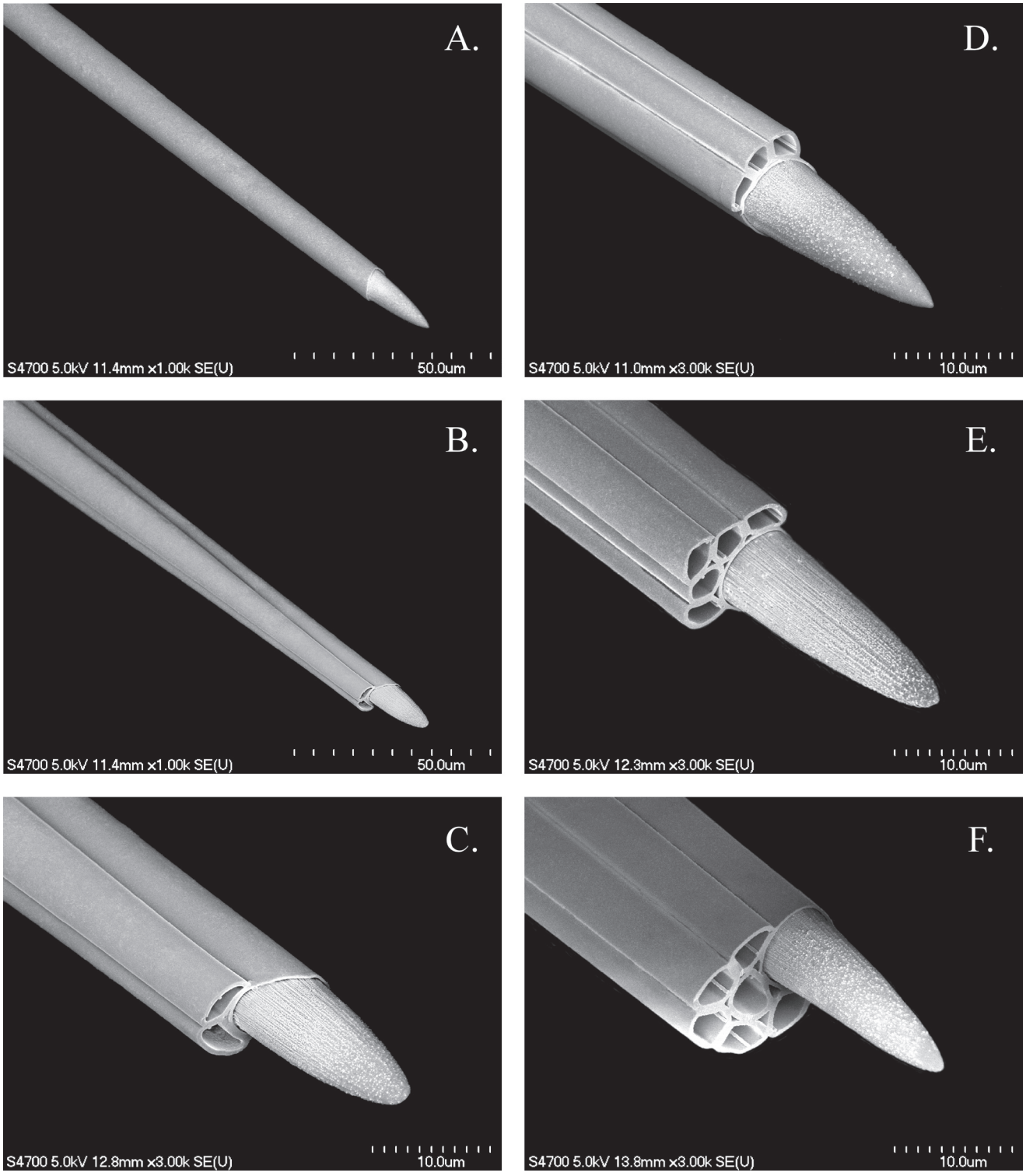

Figure 1. Scanning electron micrographs of carbon fiber microelectrodes. Single-barrel microelectrodes are consisted of a conical carbon tip protruding from the borosilicate glass insulation (A). A varying number of micropipettes can be attached to the recording carbon fiber containing barrel (B-F) for delivering drugs by microiontophoresis or pressure. Filling of the drug barrels is facilitated by inner glass microfilaments fused to the inner wall of the microcapillaries (F).

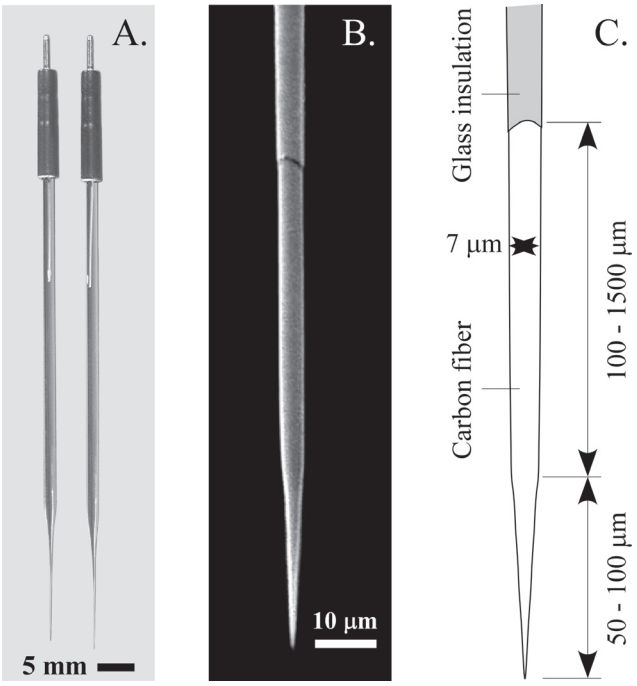

Figure 2. Macroscopic (A) and scanning electron microscopic (B) view of single-barrel carbon fiber microelectrodes made for submicron-scale electrophysiological, electrochemical or biosensor applications. Section of the carbon fiber protruding from the glass insulation (B,C) allows shaping of submicrometer tips. The exposed carbon surface can be modified chemically and is to be insulated by electropolymerization. Ultrasmall electroactive area can be formed on the very tip.

with a diameter of about 7 μm was glued to a 2.5 cm long 28 AWG tin-plated copper wire using a conductive paint. One end of the wire had previously been soldered into a gold-plated male connector pin. Beginning at its free end, the CF was sucked into the glass capillary tubing using gentle vacuum. The connector pin was then fixed onto the end of the glass tubing by 12 mm long heat-shrinkable plastic tubing. For single-barrel microelectrodes, this „blank" assembly was ready to be pulled. Construction of multibarrel CF microelectrodes was published elsewhere (Budai and Molnár 2001). The two ends of the electrode blank were then held by the chucks of a vertical micropipette puller (Gravipull-2, Kation Scientific, Minneapolis, MN) and a heating coil was used to soften the glass gently in its central portion. Pulling force was provided with gravity only using a variable mass system. In consequence of the very high tensile strength of the CF, it did not break during the pulling procedure. The excess length of fiber protruding from the tip of the glass assembly was cut with a fine pair of scissors to about 5 mm. The exposed CF was finally trimmed to the needed length using electrochemical etching (Armstrong-James et al. 1980; Anderson and Cushman 1981) or high voltage spark etching (Budai and Molnár 2001; Millar and Pelling 2001) under a light microscope.

Chemicals and instrumentation

Polymerization solutions contained 2-allylphenol, phenol, 2-butoxyethanol, methanol and water. Curing of epoxy compounds, dodecyl/tetradecyl glycidyl ether, bisphenol A diglycidyl ether, was carried out using diethylenetriamine, 4-aminophenyl sulfone as curing agents. Methyl ethyl ketone was used for diluent. Electrochemical etching solution was made of concentrated sulfuric acid saturated with potassium bichromate. Physiological saline, 0.9% (w:v) NaCl, was used to measure impedances of CF microelectrodes. All componds were purchased from Sigma (Saint Louis, MO) and used as received.

Tip and substrate potentials were controlled by a two-electrode DC potentiostat (Micro C, WPI, Sarasota, FL) against an Ag/AgCl half cell. The applied potentials were set externally and the measured electrode currents were collected using an NI 6221 multifunction data acquisition board placed in a personal computer and programmed with LabView 7 (National Instruments, Austin, TX). Impedances were measured using an SC-200 impedance meter (Kation Scientific, Minneapolis, MN) applying 1 KHz (or 110 Hz above the 20 MΩ range) oscillating current. Electron microscopy was carried out using an S-4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan). Electrode samples for electron microscopy were coated with conductive films of gold with the aid of a sputter coater model SC7620 (Quorum Technologies, East Sussex, UK).

Results and Discussion

Single- and multibarrel CF microelectrodes

Carbon fiber microelectrodes made by us for extracellar spike recording and microiontophoresis are shown in Fig. 1. In its simplest form, a CF microelectrode consists of a carbon tip protruding from the borosilicate glass insulation (single-barrel CF microelectrode, Fig. 1A). View of a complete single-barrel microelectrode is shown in Fig. 2A. Electrical signals from the carbon tip leave the microelectrode through a gold-plated pin located on the top end. When extracellular spike recording is to combine with drug delivery by iontophoresis or pressure, the appropriate number of microcapillary pipettes can be attached to the recording carbon fiber containing barrel (Figs. 1B-F). Conical carbon tips of these microelectrodes were formed using high voltage spark etching (Budai and Molnár, 2001). Filling of the iontophoresis barrels is facilitated by inner glass microfilaments fused to the inner wall of the microcapillaries (Fig. 1F). CF microelectrodes of these types provide high quality extracellular spike recordings (Budai 2004) and allow testing drugs by iontophoretic delivery into the close vicinity of neurons (Budai et al. 1998).

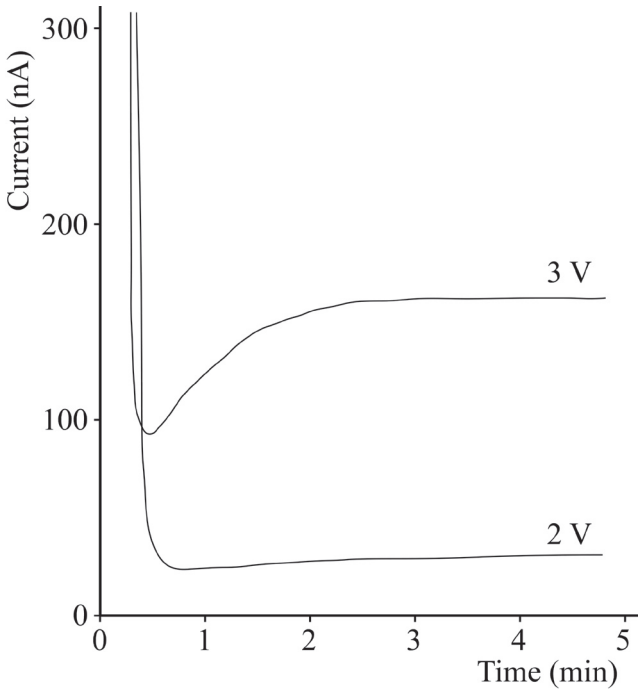

Figure 3. Current response during the electropolymerization of poly(oxyphenylene) on the carbon surface of the microelectrodes at two different holding potentials. The dip in the response at 3 V is due to a higher steady-state current eventually reached and may reflect uneven polymer formation.

Preparation of poly(oxyphenylene) insulated CF microelectrodes

The thickness of the glass insulator layer of our CF microelectrodes is about 1 μm and the carbon tip protrudes by about 20 μm from the glass assembly (Figs. 1 and 2B). Functionalization of the CF for electrochemical or biosensing applications in ultrasmall volumes requires longer sections (usually hundreds of micrometers) of the exposed, uninsulated CF where submicron tips are shaped and chemical modifications are made (Figs. 2B,C). Following these modifications, a new electrical insulation must be applied on the carbon surface. In our procedure to form an electrically insulating polymer, a thin layer of poly(oxyphenylene) was electrochemically deposited on the carbon surface of CF microelectrodes shown in Fig. 2B. These electrodes had carbon fibers protruding from the glass insulation by 1000 μm including a 100 μm-long section tapering into a submicron tip (Figs. 2B,C). The freshly made polymerization solution consisted of 2.6 ml 2-allylphenol, 0.53 ml phenol, 2.62 ml 2-butoxyethanol and 3.0 ml allylamine in a total volume of 100 ml of 1:1 (v:v) water-methanol mixture (Strein and Ewing 1992; Clark et al. 1997; El-Deen et al. 2006). Electrodeposition was carried out at room temperature using DC voltages in a two-electrode single-compartment electrochemical cell. An Ag/AgCl half-cell was used as reference electrode. After applying anodic current through the carbon fiber electrodes using 2 V DC deposition voltage for about 5 min, the electrodes were rinsed in warm 1:1 water-methanol mixture and the polymer was cross-linked by heating at 150ºC for 2 hours. Increasing the deposition voltage led to a dip in the current response due to a higher steady-state current eventually reached (Fig. 3). The higher steady-state response values may correspond with the uneven polymer formation (Strein and Ewing 1992).

The polymerization procedure consists of two steps: electrochemical generation of an electronically conducting polymer followed by curing at elevated temperature during which the deposited polymer layer becomes insulating. The *o*-allyl group on the monomer facilitates deposition of thicker films and also serves as a crosslink with thermal curing. After finishing the thermal curing, impedances of the poly(oxyphenylene)-coated CF microelectrodes were measured in physiological saline using a Ag/AgCl reference electrode. An average 17.1 ± 2.8 MΩ (mean ± S.D., n= 22) impedance was obtained for 22 electrodes. This value is significantly higher than the 0.4-0.8 MΩ impedance usual for the uninsulated CF microelectrodes (Budai and Molnár 2001).

Additional insulation by epoxy resin

To improve the insulating properties of the polymer layer, an additional epoxy coating was applied. Poly(oxyphenylene) coating was electrodeposited on CF microelectrodes as described above but using 2V DC voltage for 2 min. After rinsing the electrodes in warm 1:1 (v:v) water–methanol mixture, thermal cross-linking was partially carried out for 15 min at 150ºC and the electrodes were dipped in 20:1:1 (v:v:v) methyl ethyl ketone-dodecyl/tetradecyl glycidyl ether-bisphenol A diglycidyl ether epoxy mixture. Another curing period was followed at 150ºC for 15 min and, lastly, the electrodes were dipped in 20:1:1 (v:v:v) methyl ethyl ketone-diethylenetriamine-4-aminophenyl sulfone mixture of curing agents. Final polymerization was carried out at 150ºC for 2 hours. The additional epoxy layer significantly increased the effectiveness of the insulation of the CF microelectrodes as the impedance for each of the 38 prepared electrodes was higher than the upper range limit (200 MΩ) of our impedance meter. The thickness of the insulating layer was less than 1 μm as estimated by light and scanning electron microscopic studies.

Re-etching of the carbon tip

Following insulation of the carbon fiber by coating with combined poly(oxyphenylene) and epxy resin, removal of the polymer from the exact tip of the electrode was accomplished in two ways. For extracellular recordings, high voltage spark etching was applied using a polished gold tip as counter electrode (Budai and Molnár 2001). We have found this method of polymer removal to give an assumedly small electroactive

area as the impedance of these electrodes averaged at 1.3 ± 0.3 MΩ (mean ± SD, n= 13). For biosensing purposes, the insulating polymer needs to be removed from a longer section of the carbon tip. So, the electrodes were placed in a drop of electrochemical etching solution under a light microscope and an AC voltage of 3 to 6 V was applied for several seconds (El-Deen et al. 2006). The length of polymer removal was controlled using the fine movement of the microscope's stage. Impedances of these microelectrodes ranged from 0.6 to 1.1 MΩ.

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