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Perspectives

Neuro-vascular central nervous recording/stimulating system: Using nanotechnology probes

Rodolfo R. Llinás¹, Kerry D. Walton¹, Masayuki Nakao², Ian Hunter³ and Patrick A. Anquetil³ ¹Department of Physiology and Neuroscience, NYU School of Medicine, 550 1st Av., New York, NY 10016, USA (Tel.: +212-263-5415; E-mail: llinar01@popmail.med.nyu.edu); ²Institute of Engineering Innovation, University of Tokyo, Japan; ³Department of Mechanical Engineering, BioInstrumentation Laboratory, Massachusetts Institute of Technology

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Abstract

Electrical recording from spinal cord vascular capillary bed has been achieved demonstrating that the intravascular space may be utilized as a means to address brain activity with out violating the brain parenchyma. While the initial demonstration was implemented using electrically insulated platinum electrodes *in vitro*, the possibility of using conducting polymer filaments is now being explored. This paper presents a set of highly possible future scenarios where the integration of electrophysiology and novel polymer technology may serve as a new approach towards basic and medical neuroscience.

Introduction

When considering the role of neuroscience in modern society the issue of the brain-machine interface is one of the very relevant problems to be addressed in the next two decades. Indeed, our ability to design and build new information analysis and storage systems that are light enough to be easily carried, has advanced exponentially in the last few years. Ultimately, the brain-machine interface bottleneck will become the major stumbling block to robust and rapid communication with these devices.

To date, the interface improvements have not been as impressive as our progress in miniaturization or computational power expansion. Indeed, the limiting factor with most modern devices relates to the human interface. Buttons must be large enough to manipulate, screens wide enough to allow symbol recognition, and so on. Clearly, the only way to proceed is to establish a more direct relationship between the brain and such devices. And so, the brain-machine interface restriction will indeed become one of the central issues of modern society. As this is being considered, another quite different revolution is being enacted by the very rapid and exciting developments in the field of nanotechnology. Such developments deal with manufactured objects with characteristic dimensions of less than one micrometer. This issue is of high relevance here since the brain-machine impasse may ultimately be resolved through nanotechnology. Obviously, what is required is a robust and noninvasive way to tap and address brain activity, that is an optimized cognitive based command/control.

In addition to serving as a brain-machine interface, such an approach would be extraordinarily valuable in the diagnosis and treatment of many neurological and psychiatric conditions. The technology to be described here will be vital in the diagnosis and treatment of abnormal brain function. Such technology would allow constant monitoring and functional imaging as well as direct modulation of brain activity. For instance, an advanced variation of present day deep brain stimulation would be of excellent therapeutic value. Besides, interface with 'intelligent' devices would significantly improve the lives of disabled individuals allowing them to be more fully involved in everyday activity.

Recording and stimulating brain activity in animals and humans

Brain activity is mostly recorded with electrodes placed on the surface of the skull. In the case of electroencephalography (EEG), electrodes are placed on the skull and record activity occurring on the surface of the brain (Nunez, 1981). In the case of magnetoencephalography (MEG), recording probes are also placed on the surface, but through triangulation such activity can be mapped in three dimensions (Yamamoto et al., 1988; Suk et al., 1991). To record from within the brain the skull must be opened and electrodes lowered into the brain mass. Similarly, to stimulate the brain, as is done therapeutically for some patients with Parkinson's disease, the skull must be opened and electrodes inserted (Kolchinsky, 2001).

In the last two decades there has been a serious interest in multicellular recording from the central nervous system. This drive resulted from the understanding that brain activity is, fundamentally, the resultant of coherent multicellular activity to be understood only by simultaneous recording of significant number of neurons (Llinás, 1991). Indeed, devices are routinely used to conduct neuroscience research in monkeys and other animals. Mechanically, the devices must gather signals from a number of recording sites, typically arranged in a grid pattern. The need to record from an increasing number of neurons led to the development of microelectrode arrays (Bower & Llinas, 1982; Fukuda et al., 1989, 2001; Llinas & Sasaki, 1989; Chapin & Moxon, 2001; Martel et al., 2001; Donoghue, 2002; Zhang et al., 2004). Many designs and manufacturing techniques have been developed to construct these arrays (Geddes, 1972: Nicolelis, 1999; Fofonoff et al., 2004).

Use of a neuroprosthetic array requires that it be both easily manufactured (Guillory & Hatt, 2002) and biocompatible. Electrical discharge machining (EDM) is a process that makes use of computer aided design (CAD), that runs under computer numerical control (CNC), and that is capable of batch processing. It can generate intricate features with high aspect ratios and is capable of machining a large variety of conductive materials. Microelectrode arrays fabricated in our MIT BioInstrumentation Lab by EDM and chemical etching have been used to successfully record neural activity in mouse brain (Fofonoff, 2003).

However, at present, microelectrode arrays are inadequate for long-term implantation. Implantation results in the migration of activated glial cells and other inflammatory cells (platelets, neutrophils, monocytes/macrophages) to the arraytissue interface. Adherence to the array surface promotes glial scar formation and ultimately fibrous encapsulation (Massia, 2003). While effectively sealing the site of injury, encapsulation blocks neurite growth and axonal regeneration (Massia, 2003). In an effort to establish a more reliable, long term interface between microelectrode arrays and neural tissue, several investigators have used bioactive coatings to promote biospecific cell adhesion for material implant applications (Cui et al., 2003; Massia, 2003; Kim et al., 2004). While results are encouraging, the use of potentially biocompatible microelectrode arrays to monitor/stimulate neural activity is extremely invasive.

Neurovascular approach

One of the most attractive possibilities that came to mind in trying to solve the hardware problem concerns the development of a vascular approach. The fact that a rich vascular bed permeates the nervous system parenchyma makes this space a very attractive candidate for our interface (Figure 1). Gas exchange and nutrients delivery to the brain mass occurs across 25,000 m (Figure 2) of capillaries with a diameter of approximately 10 μ m (Figure 3).

The final level is depicted in Figure 4 where a light micrograph of a cerebellar Purkinje cell is shown on the left. Note the spaces for the capillary bed (c). On the right an electronmicrograph of the cerebellar cortex shows the actual capillary and the relative size of a 500 nm wire (red circle), for context. Concerning the acquisition of brain



Figure 1. Lateral view of the head and brain with radio-opaque dye showing arterial system. (Modified from the Ciba Collection of Medical Illustrations, Vol. 1. The Nervous System, Pt. 1. Anatomy and Physiology, Section III, plate A. By Frank H. Netter, CIBA, 1983).



Figure 2. Close-up view of brain arterial system showing graduate decrease in vessel size.

activity through the vascular system, the use of nanowire technology coupled with nanotechnology electronics seems very attractive. This approach will allow the nervous system to be addressed by a large number of isolated nanoprobes that would be delivered to the brain via the vascular bed through catheter technology that is now used extensively in medicine and in particular in interventional neuro-radiology (Figure 5).

The basic recording device comprises a set of nanowires tethered to electronics in the main



Figure 3. Branching of artery showing branching points.

catheter such that they will spread in a 'bouquet' arrangement into a particular portion of the brain's vascular system. Such an arrangement could support a very large number of probes (in the millions). Each nanoprobe would be used to record the electrical activity of a single neuron, or small group of neurons, without invading the brain parenchyma. Obviously, the advantage of

Purkinje Cell Capillaries



Figure 4. Left: Purkinje cell showing spaces occupied by capillaries (c). Right: Electron micrograph through Purkinje cell dendrite including capillary. Dot placed in capillary is 500 nm in diameter.

such an nanoelectrode array is that the small size would insure that it would not interfere with blood flow, gas, or nutrient exchange, or disrupt brain activity.

Electrodes

Metal. Concerning the acquisition of brain activity through the vascular system, the use of nanoscale electrode probes coupled with nanoscale electronics seems very attractive (Huang et al., 2001; Nguyen et al., 2001; Gudiksen et al., 2002). It would allow the nervous system to be addressed by a large number of isolated probes that would be delivered to the brain via the vascular bed through a catheter. Present nanoscale level electronics already outstrips in complexity what we propose. Indeed Very Large Scale Integration (VLSI) technology has out performed all previous electronic miniaturization.

To date, the finest electrodes have been pulled from glass. These microelectrodes have tips less than a micron in diameter and are filled with a conductive solution. They are typically used for intracellular recordings from nerve and muscle cells. The obvious limitation is that activity is recorded from only one cell at a time. We have obtained simultaneous recordings from over 100 individual cells using multielectrode arrays. However, this is an invasive procedure as the electrodes are lowered into the brain from the surface (Sugihara et al., 1995; Yamamoto et al., 2001).

The problem we consider has two main issues to be addressed: (i) hardware properties and limits that would allow direct and robust access to brain function and (ii) software based analysis and interpretation of brain signals. To approach these issues, we have proposed and obtained a set of 'Proof of Principle' studies for a new technology that would allow the direct interaction of a machine with the human brain (Llinas & Makarov, 2003). This nanointerface would be secure and minimally invasive.

Polymer. This new development will be addressed below.

Peripheral nervous system

Our first set of experiments initially demonstrated that recordings of electrical activity could be made from within the vascular space (Figure 6). For these experiments we utilized an electrode system comprising four 20 μ m Pt/Ir wires (total length 75 mm). Our key finding is illustrated in Figure 6 which shows the distribution of evoked electrical activity from a peripheral nerve simultaneously recorded from three intravascular electrodes (c, b, a) spaced about 3 mm from each other at increasing distances from the stimulating electrode on the activated nerve bundle (out of the picture to the right) and an electrode (d) placed in direct contact with the stimulated nerve. The averaged recordings are superimposed at the bottom of the figure and the same letters as the individual traces and color-coded arrows in the photograph indicate the recording site.

Figure 7 shows an average (Figure 7a) and two single recordings (Figure 7 b, c) made from within the sciatic artery after sciatic nerve stimulation. A portion of each recording is shown in panel 'd' at a higher gain. The noise level of the single recordings is similar in frequency but larger in amplitude than that of the average. All these are in low microvolt range indicating that blood flow and related turbulence does not constitute a serious hindrance to intravascular recording, from an electric noise point of view. A power spectrum analysis was carried out on the signals shown in panel 'd'. In order to determine the frequency characteristics of the intravascular recordings a set of power spectrum analysis on eighteen different sets of recording experiments. The power spectral densities (PSD) shown in panel 'e' were normalized to the 100% to compare the peak frequency. These recordings show remarkable consistency among the intravascular recordings and indicate that the signal to noise ratio is outstanding with signal averaging (100:1) and excellent without averaging (30:1). In fact these recordings compare very well with the best extracellular microelectrode recording in the brain parenchyma

The final figure from this set of experiments compares averages (n = 20) of traces made from the surface of the nerve (using a 300 μ m silver ball electrode) with those made from within the vessel (using 20 μ m Pt/Ir microwires) (inset, Figure 8a). The noise level encountered in the averaged intravascular (red) and extracellular (black) recordings is quite similar as shown in Figure8b. Panel 'a' shows PSD for 6 averaged extracellular and 9 averaged intravascular sets of recordings.

The frequency characteristics of the noise recorded using the two types of electrodes were

similar with the largest peaks between 11 and 14 kHz. There is a clear power difference consistent with recording electrode size. The average voltage of the signal shown in panel 'b' is $-17.96 \ \mu\text{V}$ for the intravascular and $6.878 \ \mu\text{V}$ for the nerve recording. Thus, the vascular epithelium, even of an artery, does not provide a barrier to the electrical signal. The differences we have recorded may be partly attributed to the differences in the recording surface area and on the diameter of the recording electrodes.

These findings are most encouraging as they indicate none of the expected problems concerning noise or transvascular resistivity will be a problem. More to the point, the electrical proximity to the neuronal environment may be better that with parenchyma electrodes as no neuronal injury is generated by the advancing recording microelectrode.

Proof of concept experiments

Experimental goals. The following goals were identified; (a) to develop optimal trans-vessel recording conditions, (b) to develop techniques for introducing and guiding nanoelectrodes within vessels; (c) to develop techniques for fixing electrodes in position within vessels, (d) to demonstrate that transvascular recording and stimulation, at the capillary level, is feasible and, (e) generate data that will aid in the design of nanoelectrodes. We will also specify the optimal characteristics for recording electrodes, pre-amplifiers, and amplifiers, signal averaging and other ways to optimize the signal.

Thus we designed experiments that would identify the optimal conditions for obtaining and holding a robust signal recorded across the vascular system and those factors that influence the robustness of the neurovascular approach. At the same time, we are developing a package of mathematical algorithms and a computational toolkits that would be appropriate and effective for data analysis and decision-making.

Central nervous system

A new set of electrodes was developed for the second set of proof of concept experiments. Professor Masayuki Nakao in the Institute of



Figure 5. Using an intravascular catheter to introduce and deliver nanoprobes to the brain.



Figure 6. Sciatic artery and nerve with intravascular (a, b, c) and surface extracellular (d) electrodes and responses eleicted by sciatic nerve stimulation.



Figure 7. Comparison of singles and average recordings from within the sciatic artery after sciatic nerve stimulation.



Figure 8. Comparision of intravascular and extracellular nerve surface recordings.



Figure 9. Design of electrode and fluid delivery system.

Engineering Innovation at the University of Tokyo, Department of Engineering Synthesis manufactured this new form of nanoscale electrodes. Dr. Nakao and his department have been making nanoscale devices and supporting electronics for research and for clinical use in human patients for 10 years. The design of the micromanipular, catheter and electrode used in these experiments (constructed by Dr. H. Takahasi and Mr. H. Watanabe) is shown in Figure 9.

Each electrode array comprises 2-4 insulated wires of different lengths. Each wire within the array ends in a small, bare, cup-like enlargement (Figure 9e). This enlargement is covered with platinum black which provides a necessary impedance reduction agent and acts as a 'sail' to help move the electrode within the vessel. Thus, once the electrodes are in the vessel, they are carried by the infusion of Ringer's solution and/ or the blood stream until they extend to their full length. We have implemented single $0.6 \,\mu m$ diameter electrodes and are planning to proceed with small arrays of electrodes. One aim of these new experiments is to determine the optimal electrode design for recording and extension within the vessel.

Electrode visualization. In these experiments, nanoelectrodes are inserted into mesentery vessels near a nerve bundle or plexus. The electrodes are introduced into the vessel through a catheter that is tied in place. The electrode is then advanced and carried into the vessel by an infusion of Ringer's solution. This preparation is transparent and progress of the electrode in the vessel can be followed using a video camera. This allows evaluation of the mechanical properties of the electrodes and their behavior at vessel branch points.

CNS recordings

More recently in collaboration with Dr. Walton, assisted by Mr. Watanabe, we have been able to record from the spinal cord in vitro at NYU. These are very significant results as they demonstrate the possibility to record CNS activity across a vessel in spite of the 'high resistance' of the vessel wall. In fact the results obtained give a larger signal from the intravascular space than from the CNS extracellular milieu (Figures 10–12). The clarity of the responses and the lack of overwhelming noise indicate, indeed,

that this route may be the most propitious for addressing CNS activity.

In short, the present set of results demonstrates that intravascular electrical recording and or stimulation can be implemented across capillary vessels in the CNS or via the peripheral vascular system. This technology will be greatly enhanced by substituting metal nanowires with polymer nanofibers that will serve as flexible nonreactive anisotropic conductors (high electrical conductivity along the fiber, very low radial conductivity obviating insulation requirements) down to 200 nm in diameter that can be permanent or biodegradable, depending on the polymer used.

Indeed in Figure 12b a set of recordings obtained intravascularly following sciatic nerve with $15 \times 15 \ \mu m$ conductive polymer strip from the I.W. Hunter laboratory compares favorably with those obtained with silver micro-wires (Figure 6).

While present results concern CNS electrical recordings, preliminary data (not shown) indicate that CNS electrical stimulation can also be implemented using similar nanotechnology.

The conducting polymer nanowire approach

We are presently working to substitute the platinum electrode wires used in the above experiments with



Figure 10. Mid-spinal vessel recording using a 1 μ m intravascular electrode. Comparing extracellular and intravascular recording as the stimulus amplitude is increased. Amplitude in mV, time in ms.



Figure 11. Intravascular recording in the spinal cord. Note that the intracapillary electrodes have similar noise levels, the amplitude for the field potential are larger and are more differentiated. 'a' and 'b' amplitude change with increase stimulus magnitude. Single traces. In 'f' N2 P3 waves indicate synaptic (cell–cell) communication recorded.

conducting polymer nanowires. In the following sections we introduce conducting polymers, discuss their current properties and detail the approaches we are currently taking to fabricate sub-micrometer diameter conducting polymer nanowires. Of interest is also a contractile form of the conducting



Figure 13. Common conducting polymers. Note the alternation of single and double bonds along the polymer backbone.



Figure 12. A second set of recordings to compare directly extracellular an intravascular at increasing stimulus strengths. Figure 12b as in Figure 6 but using a polymere intravascular electrode. Upper left panel $15 \times 15 \,\mu$ m thick polymer strip electrode. Upper right panel recording arrangement. Lower panels intravascular recordings (two different experiments) following sciatic nerve stimulation.



Figure 14. Stacked molecular conformation of PPy derived from X-ray analysis (Lee, 1999).



Figure 15. Electrochemical redox cycle for polypyrrole. A⁻ represent anions, and e⁻ electrons.

polymer material, allowing one to create a steerable version of the nanowire. We conclude with an analysis of biocompatibility issues and the importance of developing biodegradable conducting polymer wires for short term brain implants.

Polymers, e.g. plastics, are a commodity of modern life. Their use is ubiquitous from low tech, high volume consumer products such as packaging materials, tubing or furniture to high tech specialized products such as medical implants or technical garments. Typically one thinks of polymers as insulators. Indeed, a typical polymer such as polyethylene (PE) has a conductivity of 10^{-16} S/m, which is 22 orders of magnitude smaller than copper $(5.9 \times 10^6 \text{ S/m})$. This common picture however changed in 1977 when Alan MacDiarmid, Alan Heeger and Hideki Shirakawa made the first discovery of a conducting polymer, polyacetylene: $(CH)_x$. By adding doping elements such as Br₂, I₂, AsF₅ to polyacetylene they discovered that this relatively insulating polymer (typically about 10^{-6} S/m) could be either n- or p-doped and ultimately show high intrinsic conductivities (up to 10^7 S/m) (Ito et al., 1974; Shirakawa et al., 1977). This discovery led to a rapid growth and intensive study of a novel class of organic materials: synthetic metals.

The surprising property of synthetic metals is that their conductivity shows metallic behavior, although they contain no metallic element. On the contrary, the inherent conductivity of these materials is the result of the conjugated structure of their molecular backbone. The bonds between carbon atoms that form the conjugated polymer backbone are alternating single and double bonds. As a result, these are composed of both strong σ bonds as well as delocalized π bonds which exhibit a weaker molecular force. Figure 13 shows common conducting polymers: polyacetylene, polyaniline, polypyrrole, polythiophene and polyethylene dioxythiophene (while Figure 14 shows the stacked molecular structure of polypyrrole). It is important to note that Figure 13 represents only a tiny fraction of the members of the conducting polymer class of materials, most of which have yet to be synthesized.

Addition of a charged molecule, a dopant, allows displacing the weakly bound π electrons from the backbone and ultimately leads to the conduction properties of these plastic materials. As dopants are added to the polymer, electrons or holes (a hole is an abstract charge, which corresponds to a position where an electron is missing) have to be injected to maintain charge neutrality. Charge transport inside the polymer takes place both along the backbone (intrachain transfer) as well as from chain to chain (interchain transfer), both via a so-called hopping mechanism. Recent studies using quantum mechanical theory calculations backed by ultra fast spectroscopy indicate a two-step mechanism for intrachain energy transfer with hopping along the conjugated chains as the rate-limiting step (in the range of 1 ns^{-1} ; the higher efficiency of the interchain transfer process, they argue, is mainly due to larger electronic coupling matrix elements between close lying chains. A goal in the conducting polymer community has been to develop polymers with stronger π - π interacting building blocks, thus creating higher motilities and, possibly a truly delocalized transport regime similar to metals (Sirringhaus et al., 1999).

Since the discovery by Shirakawa, it has been found that conducting polymers not only feature unusually high conductivities, but also are excellent materials for energy storage (high energy density batteries and supercapacitors), energy/information shunting (transistors), displays (LEDs and electrochromic devices), sensors (strain gauges, photodetectors and chemical sensors) and actuators. Conducting polymers offer the designer a greater range of capabilities than silicon. It is clear that in the near future integrated electro-chemo-optomechanical devices will be constructed entirely from conducting polymers, at very low cost.

The doping process

The extended π -system of conjugated compounds is ultimately responsible for the generation of

mobile carriers. In the states shown in Figure 13 the charge carrier delocalization is incomplete and the polymers are hence semiconductors. The oxidation levels have to be changed to lead to the creation of states between the conduction and valence bands. Note that in several polymers, the band gap can be completely eliminated upon full

oxidation. As mentioned previously the polymer oxidation states can be changed via doping, analogous to traditional semiconductors such as silicon and germanium. The doping process may be achieved chemically, electrochemically, and by photon absorption. Of high interest to this work is that the doping level and hence the conductivity can be chemically and electrochemically switched. In the case of electrochemical doping, the material's oxidation state (doping level) can be switched continuously from a semiconductor to a metal via an electrical signal. This process is schematically depicted in Figure 15 and Figure 16. Note that doping is typically reversible, and we and others have been able to fabricate conducting polymer transistors which have electrochemically controllable resistance. A number of other properties change with doping level, including color and volume. The volumetric changes are made use of in traditional actuators such as polypyrrole (PPy) and polyEDOT. A set of polymer wires has also been fabricated (Figures 17, 18).

Conducting polymer synthesis

The conducting polymers presented in this work are synthesized via electrochemical deposition onto a conductive electrode (e.g., a working electrode). Electrochemical polymerization allows precise control of polymer growth via the amount of electrons passed through the electrical circuit. The reaction taking place at the working electrode during the synthesis of polypyrrole is described below: with n > 2 and where e⁻ are electrons and A⁻ the anion (or counterion) necessary to balance the charge on the polymer as it is grown. The counterion is incorporated at the time of synthesis and is intercalated between PPy chains. Elemental analysis shows that dry films have an oxidation state of 0.3 charges per monomer, hence the n/3factor in the above reaction equation. Polymerization is believed to occur via chain growth coupling of oxidized monomers in radical cation form. Polymerized PPy oligomers eventually precipitate on the working electrode out of solution once a solubility limit is reached.

The electrochemical setup and a typical resulting film are presented in Figure 17. It consists of a working electrode onto which the polymer is deposited, a controlled source of current (or galvanostat) an electrolyte, a solvent and a counter electrode to close the electrical circuit.

Conducting polymer microwires

The conducting polymer microwires are manufactured via slicing electrochemically grown polypyrrole films. Figure 18 below shows electron micrograph images of some of our conducting polymer wires manufactured via this process. A 15 μ m thick polypyrrole film placed onto a microtome was sliced into 2 μ m thick wires.

Production of conducting polymer nanowires

We are working on a number of nanowire fabrication approaches.

Deposition on to a fibrous template

One of the more obvious fabrication methods to produce conductive polymer nanowires is to coat existing polymer fibers of the appropriate dimen-

sions with a conductive material. As the template must be long (\sim 1 mm) and well below one micrometer in diameter, the set of usable templates is confined to two types: electrospun polymers and carbon nanotubes. Fiber coating procedures are currently under development by several members of our MIT BioInstrumentation laboratory, including Bryan Ruddy, Rachel Pytel and Naomi Davidson.

Electrospinning allows the production of fibers under 100 nm in diameter from a wide range of polymers, including biopolymers such as silk (Sukigara et al., 2003). Silk is a particularly promising substrate, as it is strong and tough, yet also biocompatible. An insulated conducting polymer wire can be produced by chemical polymerization on the fiber, either in solution (Dong et al., 2004) or in vapor (Cho et al., 1997), followed by subsequent application of an insulating material. Alternatively, carbon nanotubes can provide an extremely strong, highly conductive template for use in more permanent applications. Nanotube fiber template of the appropriate length and diameter range can be produced by spinning, by lithographic patterning (Hata et al., 2004), or by use of individual largediameter nanotubes (Hughes et al., 2004). Carbon nanotube templates can potentially be used to produce conducting polymer wires by electrodepositing the desired polymer on the fiber, then applying an insulating coating.

Application of conventional plastics processing techniques to processable conducting polymers

There is a library of conducting polymers that are soluble in organic solvents and can be melt-processed, including polyaniline, substituted polythiophenes and substituted polypyrroles. Rachel Pytel and Nathan Vandesteeg of our MIT BioInstrumentation Lab are currently investigating these processing techniques. While these materials are somewhat less conductive than intractable polypyrrole, they nevertheless have more than adequate conductivity for the brain interface applications. Polyaniline is significantly less processable than the substituted conducting polymers, but is still interesting due to its potential for biocompatibility. Intelligent nanostructured scaffolds have been created by covalent attachment of the laminin adhesive peptide, YIGSR, onto the surface of polyaniline films/fibers and

into the polymer structure during synthesis (Guterman-Tretter, 2003).

One way to produce all-polymer insulated wires with these processable materials is to draw the wires from a preform, in the same manner as optical fiber. It has been demonstrated (Bayindir et al., 2004) that this process can yield repeatable feature sizes down to 100 nm, and uses fiber drawing techniques to produce dielectric waveguide fibers. Using similar methods, a complex polymer wire preform could be produced, possibly with multiple conductors and/or shielding, and drawn down to the desired size. Since fiber drawing generally produces fibers larger than 100 nm, the preform would likely need to include removable filler material and/or multiple wires. With care, this technique could also potentially produce wires of varying diameter, similar to the platinum electrodes already tested.

As a variant of the previous technique, a conducting polymer that has a processable precursor could be used. For example, poly (*p*-phenylene vinylene) (PPV) is prepared during thermal conversion of a highly processable precursor polymer (poly[*p*-xylene-alpha-dimethyldulfonium chloride]; PXDMC) (Figure 19). When films (14 mm × 50 mm × 20 μ m) are heated above 115°C, the elimination reaction shown below occurs, converting the insulating precursor to a conductive polymer.

Volatile gases emitted during the reaction plasticize the film, allowing it to be drawn to high elongations, allowing control of the degree of structural order and film morphology. Film conductivities have been shown to increase from 4×10^3 S/m for unstretched film to 2.8×10^5 S/m for films with a draw ratio of 9. Draw ratios of 20 have been reported in the literature. X-ray analysis confirms the high degree of alignment, with a Herman's orientation parameter of 0.98 for a draw ration of 8.5 (Granier et al., 1986; Gagnon et al., 1987).

Alternatively, the solubility of these conducting polymers allows for fabrication by electrospinning. As the fluid properties of conducting polymer solutions are not normally amenable to electrospinning on their own, a core-shell technique is required. This technique spins the conducting polymer solution as the core of a jet of a more electrospinnable fluid. The resulting wire contains a conducting polymer core totally coated with insulating material, and is within the appropriate diameter range for our application. To allow the production of a long wire rather than a fibrous mat, the electrospun fiber can be directed at a rotating drum electrode. For both of these processing techniques, the insulation needs to be soluble in a solvent different from the conducting polymer solvent in order to allow external connection to the wires and to allow tip modifications.

Electrical properties of conducting polymers

Our conducting polymer wires exhibit metallic-like conductivity. Figure 20 below shows the highly linear relationship (i.e. Ohm's law) between voltage and current which is typical of our conducting polymer wires. The slope of this linear relationship is the resistivity of the material (the inverse of resistivity is conductance).

The first thing to observe is that Ohm's law (i.e., current is proportional to voltage) is followed up to very high current densities. Indeed in the experiment shown in Figure 21 current flowed for 15 s before the polymer burned at 13 MA/m^2 . By comparison the usual upper working limit of copper wire in a high performance electric motor is 10 MA/m^2 . The conductivity of the conducting polymer grown for this experiment was 37 kS/m (i.e. the slope of the current density verses electric field plot below). A conducting polymer wire does not need to have sub-micrometer diameters until near its tip. Consider a wire having a diameter which is progressively stepped from 100 μ m down to 200 nm. If the 200 nm diameter tip region extends for 100 μ m then such a wire if made from a conducting polymer having a conductivity of 37 kS/m would have an overall resistance of less than 10 k and could carry currents in the order of 500 nA. We expect that the conductivities of our conducting polymers will increase considerably as we learn to improve their method of manufacture.

It is expected that these nanowires will have superior resistance to fracture in the brain nanowire application. Malleable materials such as gold or platinum will require minimal forces to permanently deform the wires with very small diameters. Conducting polymers are not malleable, and should therefore be more resistant to deformation due to impact with particulates in the blood. Conducting polymers are typically tested at low voltages (e.g., < 10 V). However if they are to be used for brain stimulation (as well as recording) it is essential that they survive high voltages (e.g., 100 V). We have recently shown that our conducting polymer wires do degrade when subject to 160 V pulses.

Mechanical and electrical testing apparatus

We have built a nanoscale fiber mechanical and electrical testing apparatus (Figure 22) that is capable of performing a variety of 1D mechanical measurements on fibers ranging from 50 nm to 20 μ m in diameter. This instrument will be used to determine if the polymer nanowires are more robust than comparably sized metal wires.

The fiber to be tested is attached between a computer controlled micro-actuator and a highly sensitive force sensor. A software interface allows the user to impose a wide variety of mechanical length perturbations to the fiber while measuring the force developed in the fiber.

The micro-actuator consists of a compound linear motor and piezoelectric transducer that together allow a wide range of strain states to be imposed upon the fiber or nanowire that is being tested. The linear motor (Aerotech, MX80) allows the imposition of large scale displacements of up to 50 mm at speeds of up to 1.5 m/s, while resolving the position of the linear motor stage to 20 nm resolution. Simultaneously, the piezeoelectric motor (PhysikInstrumente P-841.10) can impose small displacements (15 μ m, measured to sub-nm resolution) over a bandwidth of 1 kHz. The capability of these two motors allows a wide variety of mechanical tests to be performed, from very slow stretching over large displacements to high frequency dynamic stiffness measurements at small displacement.

The other end of the fiber is attached to custom force transducer consisting of a silvered quartz cantilever with high resonant frequency (>1 kHz.) The displacement of the cantilever is measured to about 1 nm resolution using a twin-wavelength laser interferometer (Hewlett-Packard). Different micro-machined beams (i.e. having differing beam deflection stiffnesses) are used for to cover different force ranges.

As an example consider a 200 nm diameter nanowire fabricated out of a conducting polymer



Figure 16. Molecular space-filling model of the electrochemical redox cycle for polypyrrole. (a) Reduced state. (b) Oxidized state.



Figure 17. Schematic electrochemical synthesis setup cell (a) and resulting PPy film (b). The films were $27 \ \mu m$ thick, 20 mm wide and approximately 160 mm long. The film side shown here has a shiny appearance, which is characteristic for the crucible side of PPy films.



Figure 18. Electron micrographs of conducting polymer microwires. The wire in the top image has a 15 μ m square cross-section with a total length (not shown) of 20 mm. The wire in the bottom left image is a close up of the wire above it. The wire in the bottom right image has a cross-section of $15 \times 2 \mu$ m.



Figure 19. Conducting polymer.



Figure 20. Current–voltage relationship in conducting polymer microwires.



Figure 21. The current density flowing through our conducting polymer microwire as a function of electric field over a large range of current densities.



Figure 22. Nanoscale fiber testing apparatus.



Figure 23. Two still frames of a video showing a conducting polymer trilayer actuator moving between two positions.

having an elastic modulus of 800 MPa (which is typical). A force of 2.5 nN is required to stretch this wire by 0.01%. Such forces are readily measured using our apparatus.

In addition to being quite robust, conducting polymers are an attractive alternative to precious metals for a variety of reasons. In our MIT BioInstrumentation Laboratory, high quality macroscopic polypyrrole is routinely produced at a cost of approximately \$2/kg. Alternatively, platinum foil of similar dimensions cost approximately \$30,000/ kg (VWR international). While there is some cost increase in the technology development to make very small wires, the cost advantage of using organic material instead of metals remains. Conducting polymers have a density similar to brain tissue which is about 20 times less than platinum.

Conducting polymer actuators

In a particular form, conducting polymers can be used as actuators. Such actuators feature muscle-like contractile properties at very low drive voltages (\sim 1 V), while being capable of generating high stresses up to 40 MPa in its linear form (cf. 0.35 MPa mammalian skeletal muscle) (Anquetil, 2004). Figure 23 below shows a trilayer actuator constructed in our laboratory, capable of generating large displacements.

We are developing a steerable form of the conducting polymer nanowires. This would allow us to steer the nanowire-probe selectively into desired blood vessels, thus creating the first true steerable nano-endoscope.

Conducting polymer biocompatibility and biodegradation

For brain interface applications it is advantageous for the wires to be biocompatible and biodegradable. The use of conducting polymers as biomaterials is attractive because it allows the coupling of electrical stimulaton and cell growth/modulation. Biocompatibility is influenced by several factors including the free energy at the solid–liquid interface, the hydrophobic/hydrophilic character of the surface, and the surface chemistry/charge density. Neutral polymers and polyanions appear to be less cytotoxic than polycations. Polymer flexibility, surface roughness, and molecular weight have also been shown to influence biocompatibility. Low molecular weight polymers absorb less protein and display less platelet adhesion. (Wang et al., 2004).

Contact between blood and a biomaterial results in a rapid activation of the coagulation and complement systems (Hong, 2004). Within a millisecond of contact, a layer of protein adsorbs to the material. Fibrinogen, which in the presence of thrombin, forms the fibrin scaffold, also acts as an adhesive, causing platelets (PTLs) to attach to the surface. Otto et al. (2004) have demonstrated that PTL adherence is influenced not only by protein preadsorption but also by blood flow conditions.

While thrombin and other activated clotting factors may be diluted under high blood flow conditions, insertion of a nanowire may alter blood flow and/or cause turbulence that could promote adhesion of PTL. This is currently of little consequence in our initial studies as the blood contact time is minimal. However, it does require consideration for any long-term studies.

Although many polymers are considered to be biocompatible, not all are degradable. Degradation or dissolution changes the shape, size, or mass of a polymer – the polymer erodes. Erosion in a physiological environment (bioerosion) can be due to solubilization of intact polymer or chemical degradation (Kohn et al., 2004). The most common cause of bioerosion is chemical degradation, primarily hydrolytic cleavage of the polymer backbone. While hydrolysis is the most common mode by which polymers degrade, oxidation of polymers (by the host or by the device or external environment) can also occur as can degradation due to enzymatic mechanisms (Coury et al., 2004). Enzymatic, cellular, or microbial degradation of polymer is referred to as biodegradation. Several factors have been shown to affect bioerosion rate (Kohn et al., 2004). Understanding the behavior of degradable polymers will facilitate successful manipulation through better design.

The use of conducting polymers as biomaterials is attractive because it allows the coupling of electrical stimulaton and cell growth/modulation. For the most part, attempts to copolymerize have resulted in nonconducting copolymers. However, Rivers et al. (2002) synthesized a biodegradable electrically conducting polymer by 'tethering conductive pyrrole-thiophene oligomers with degradable ester linkages using an aliphatic linker'. The ester linkages can be cleaved by cholesterol esterase, an enzyme secreted by cells during normal wound repair processes and the aliphatic linker provides flexibility in between the rigid pyrrole-thiophene oligomers. The pyrrolethiophene oligomers appear to be sufficient for conductivity (Rivers et al., 2002). Polymers began to degrade, as measured by tissue infiltration, 14 to 29 days after sub-cutaneous implantation rats. The oligomers remaining in after polymer degradation should be consumed by macrophages.

More recently, Wang et al. (2004) have shown in vitro that a PPy/poly(D,L-lactide)(PDLLA) composite is capable of acting as a bioconductor. This biodegradable electrically conductive composite delivered continuous current within 0.6 to 400 μ A in a simulated biological solution for up to 8 weeks. It is expected that the acidic microenvironment created by hydrolysis of the PDLLA should increase the conductivity of the PPy with significant loss of conductivity being restricted to the final stage of degradation. Increased acidity may be problematic in vivo. Finally, the mechanical properties of such materials have not yet been fully explored, but these polymere composites are promising due to their biological significance. We are currently investigating the potential for drawing and electrospinning such biocompatible and biodegradable nanofibers.

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