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Micromachined Interfaces to the Cellular World

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This paper reviews efforts over the past three decades to apply lithography and micromachining to devices capable of interfacing with intact neural networks on a chronic basis. These efforts have mirrored developments in sensors generally and have led to advances in micromachining, on-chip circuitry, lead structures, and chip-level encapsulation. Using diffused boron etch-stops and a single-sided bulk process, two-dimensional electrode arrays can be batch fabricated and used to record extracellular multipoint single-unit neural activity over periods of several months. Similarly, stimulating probes can be used to activate neural systems with high spatial resolution and precise control of the injected charge. Using on-chip signal processing circuitry to access as many as 64 sites, such probes communicate with the outside world using five-lead silicon ribbon cables while providing on-chip signal amplification, multiplexing, current generation, and self-test. Microassembly techniques allow such probes to be stacked in three-dimensional arrays on 200–400 μm centers while displacing only about one percent of the total tissue volume. The probes can also be modified to control the local chemical environment while monitoring cellular electrical activity, facilitating important progress in neuropharmacology and in a variety of therapeutic instruments.

1. Introduction

As sensors, microactuators, and microsystems (MEMS) have been developed over the past thirty years, automotive applications have been a major driver for the technology, focusing on silicon pressure sensors, flowmeters, accelerometers, and, most recently,

gyros.[1] Primary emphasis has thus been on electromechanical and electrothermal devices. However, chemical sensors, biochips, and microfluidics are now becoming a focus for intense worldwide activity. This is very much in line with the general perception that while microelectronics has been the cause of a societal revolution during the past fifty years, biomedical activities will be the focus of a similar revolution during the next fifty. Thus, activities merging MEMS with biology and cellular systems are of particular interest. In this paper, progress on micromachined devices capable of interfacing between microelectronics and intact biological neural networks (*e.g.*, the central nervous system) is reviewed. Work on these devices began in 1966 as an outgrowth of silicon etching activities associated with air-isolated integrated circuits[2] then underway at Bell Telephone Laboratories; they may have been the first sensing devices to be micromachined. Their development since that time closely mirrors progress in MEMS generally, including the use of etch-stops, on-chip signal processing circuitry, wafer-level packaging, and high-aspect-ratio dry etching.

The use of microelectrodes to record the electrical activity of neurons is one of the oldest techniques for exploring the central and peripheral nervous systems at the cellular level. Using metal and glass micropipette electrodes,[3–4] physiologists unraveled much of the operation of single neurons over thirty years ago and have traced the general functions associated with many sensory areas of the brain in both animals and man. However, as neurons have become better understood, it has become increasingly clear that understanding this basic building block of neural systems has not allowed us to understand the systems themselves any more than understanding the function of a single transistor allows us to understand a complex microprocessor. Achieving that requires at least the ability to monitor many interconnected neurons simultaneously and then to unravel their interrelationships using complex signal processing. With an increased understanding of neural systems should come the ability to deal more effectively with a variety of debilitating neurological disorders, including epilepsy. Also, the ability to record at multiple points and then insert stimulus signals on a highly localized basis should establish a foundation for neural prostheses capable of treating, in a limited way at least, disorders such as profound deafness, blindness, and paralysis. Finally, better understanding the organization of biological neural networks will likely suggest new approaches to the realization of more advanced computing systems.

There have been numerous attempts to construct arrays of microelectrodes by gluing the shanks of micropipettes or metal electrodes together. Such efforts have produced impressive recordings around single cells[5] and in cellular networks[6] but are limited in that the actual tip separations in tissue are not precisely known (the electrodes tend to splay out as they are advanced into tissue), site placements are not precise, and the assembly process is tedious. For these reasons, lithographically defined thin-film microelectrodes have been explored on a worldwide basis since the mid-1960s. The goal is to be able to monitor (or stimulate) cellular activity at many points simultaneously with high signal quality, high reliability, and minimal disturbance to the tissue under study.

2. Basic Probe Structure

The basic structure of a neural probe is shown in Fig. 1. It consists of a micromachined silicon substrate supporting an array of thin-film conductors leading from recording or stimulating sites along the shank to signal processing electronics and/or output leads at the rear of the device. These conductors are insulated above and below by stress-compensated dielectrics. As conceived in 1966, the metal electrodes projected beyond the silicon substrate at the probe tip; however, more typically they are mounted along its upper surface, allowing recording or stimulation at many points in depth. Although early studies showed that such electrodes could record single-unit activity from intact cortical tissue,[7] it was not until the discovery of impurity-based etch-stops that the batch fabrication of such

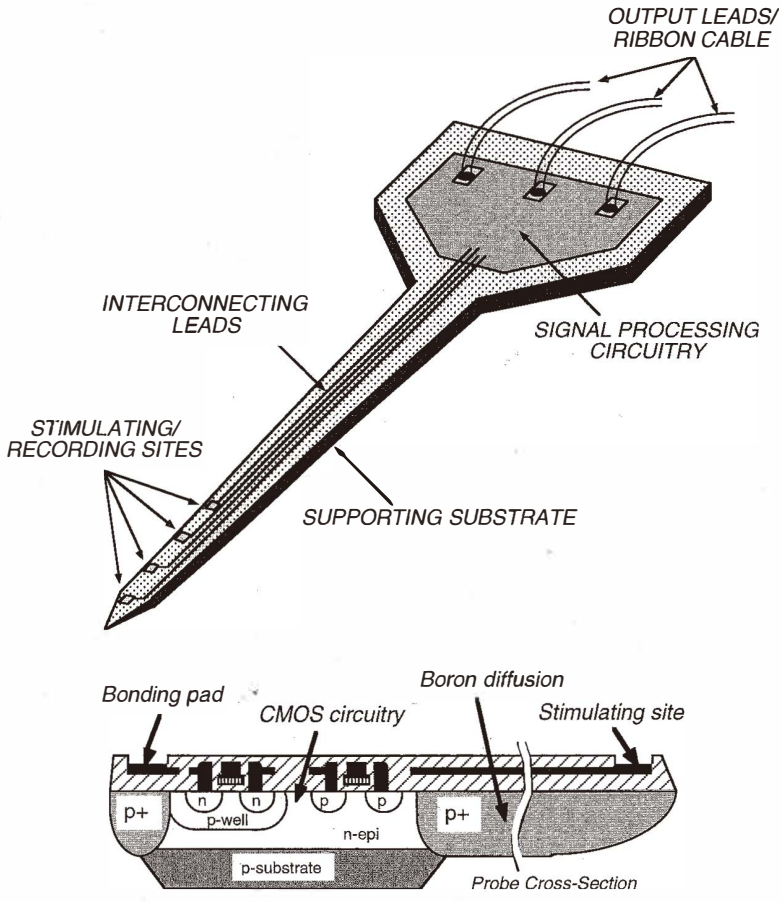

Fig. 1. Overall structure of a multisite probe for interfacing with tissue at the cellular level.

probes really became feasible.[8] With the substrate defined using deep boron diffusion, any two-dimensional probe structure can be realized, with shank widths as narrow as 20 μm controlled to ± 1 μm. Shanks vary from about 2 mm to over 20 mm in length, depending on the intended application, and are 12–15 μm thick.

The basic fabrication sequence for passive probes (with no on-chip circuitry) consists of an initial deep boron diffusion to define the intended probe area, followed by the deposition of an oxide-nitride-oxide dielectric stack. The shank conductors (polysilicon or refractory silicide) are next deposited and defined lithographically. The number of conductors per unit shank width is set by the lithographic feature size and by the number of interconnect layers used. Shank width should normally be minimized to reduce tissue displacement. In our work a pitch of 5–6 μm is normally used with one or two interconnect layers; industrially, much finer geometries could certainly be employed. Top dielectrics are deposited and contact vias are opened to define the sites and output pads. Iridium sites are used over titanium, while pads are typically gold over chromium. Contacts are opened using a two-mask process to avoid step coverage problems associated with undercutting the nitride layer. Iridium (activated to iridium oxide using cyclic voltammetry) provides an order of magnitude reduction in impedance (or an order of magnitude increase in charge delivery) compared with gold or platinum. After site and pad formation, the field dielectrics are removed, and the wafer is etched in ethylenediamine pyrocatechol to release the probes. The entire passive probe fabrication process takes seven masks and is single-sided on wafers of normal thickness. Figure 2 shows two views of a finished probe tip along with some of the two-dimensional probe designs that have been realized.

A critical problem associated with the long-term use of such structures *in vivo* is the need to get several leads to the outside world without compromising the implant. The leads are typically at megohm impedance levels, and the probe must float in tissue to avoid mechanical irritation due to motion with respect to the brain. A key to the successful chronic use of these probes has been the ability to integrate ribbon cables[9] into the basic probe structure with only one additional masking step. This step introduces a shallow (2–3 μm deep) boron diffusion to define the cable substrate. The same diffusion can be used to form sharp tips on the probes. Such cables can be realized as multilead multistrand structures in lengths of up to 5 cm or more. They are ultraflexible and have functioned well for more than one year *in vivo* and four years under 5 V bias *in vitro*.

Trends in the neuroscience community are toward larger and larger numbers of sites, and even with small features and built-in output cables, more than 64 sites on a typical two-dimensional probe is not tractable. Thus, the use of on-chip circuitry for signal amplification and multiplexing becomes mandatory for both stimulation and recording probes. It is a major advantage of this particular approach that such circuitry can be buried in the probe substrate, avoiding the need for high-density hybrid interconnects and their encapsulation. Figure 1 also shows the cross section of an "active" probe containing such circuitry. The probe is formed using an n/p epitaxial starting wafer and results in a p-type grounded silicon shield between the circuitry and the extracellular fluid. The n-epi region supports both positive and negative bias levels consistent with the creation of active electronics for current sourcing and sinking as well as amplification. The deep-boron etch-stop diffusion here is performed in two steps,[10] merging it with the active p-well drive-in from the

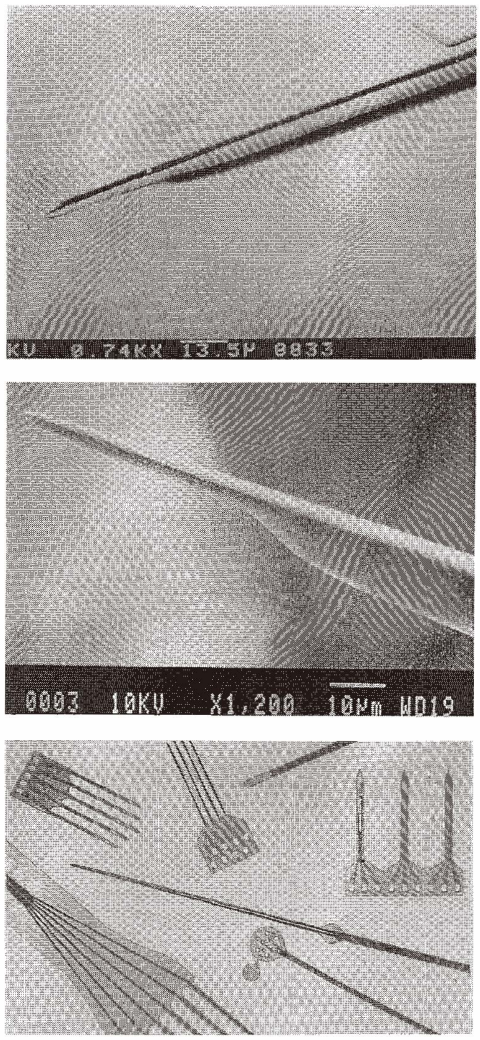

Fig. 2. SEM and optical views of neural probes. Perspective view of a probe tip (above) with a cross section (center) showing the merging of the deep and shallow boron diffusions used to define the shank. A view of several different two-dimensional probe geometries is shown below. Typical shank widths are 30–80 μm.

complementary metal-oxide-silicon (CMOS) process sequence. Otherwise, the normal CMOS process flow is undisturbed. Note that there is no etch-stop under the circuitry. Rather than use an electrochemical stop, dielectric corner compensation, simultaneous

front-back etching, and deep reactive ion etching (RIE) from the front can be used to ensure early release of the shanks and a sufficient process window to retain a thick silicon base under the circuit area.[11] A vertical RIE slot etch can also be used to eliminate lateral boron diffusion effects and reduce shank width. Shanks as narrow as 5 μm have been realized using this process.

Several families of active recording and stimulating probes have been realized using the above process and have been used successfully *in vivo*.[10–13] Figure 3 shows a set of first-generation stimulating probes. Sixty-four site active recording probes allow eight of the sites to be selected for amplification and subsequent multiplexing onto a single output line. Site selection is used to implement electronic site positioning,[13] and the on-chip amplifier provides band limiting between 100 Hz and about 10 kHz. The most recent 64-site stimulating probe selects eight sites and generates stimulus currents over a range from zero to ± 127 μA ± 1 μA. The probes generally require five leads (V_{DD}, V_{SS}, GND, data, and clock). Three-dimensional arrays can be formed by mounting several two-dimensional probes in a platform and using gold-plated lead tabs for interconnects.[14]

It should be noted that efforts to develop devices similar to those reported here are underway worldwide, and a variety of processes have been explored.[15–18] Early processes sometimes used metal foil or polyimide substrates; however, most approaches today focus on silicon due to its highly developed technology and ability to be micromachined. The silicon substrate is conductive enough to essentially eliminate interchannel crosstalk in these devices and yet does not short out naturally occurring activity *in vivo*.

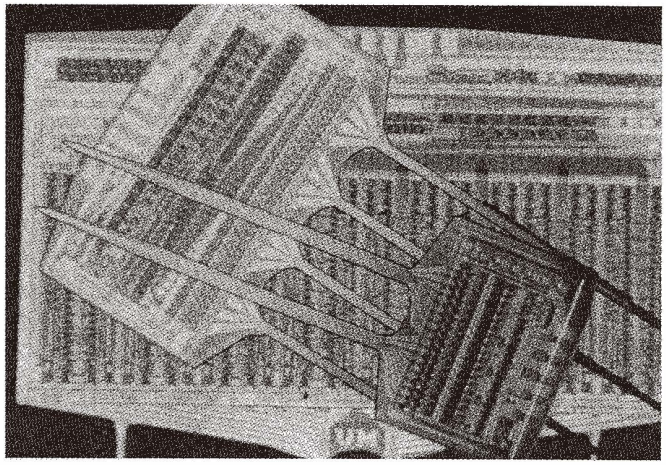

Fig. 3. View of a first-generation family of active stimulating probes. The probes contain 16 sites that can be driven in one at a time (monopolar), two at a time (bipolar), or multipolar modes. The CMOS circuitry demultiplexes a 4 MHz input data stream to select the intended site and generate the specified stimulus current.

3. Packaging Considerations

Such probes must be small enough to invade the tissue under study without excessively disturbing it and must be able to exist there without chemically degrading either the tissue or the probe. The materials present in the probe structure are all biocompatible in the sense that they do not react chemically with the tissue at an appreciable rate. However, protecting the probe from the extracellular environment so the circuitry can operate for years remains a difficult challenge. The low pressure chemical vapor deposition (LPCVD) silicon dioxide/silicon nitride dielectrics on the shanks will withstand over a year *in vivo* without noticeable degradation, and associated site impedances remain relatively stable for these time periods. In the circuit areas where bias is present, aluminum metallization is used over titanium/titanium nitride plugs. A 1-μm-thick layer of low-temperature oxide (LTO) is used over the circuitry followed by a blanket of plasma-enhanced chemical vapor deposition (PECVD) nitride.[11,19] Over this, a metal shield can be used with a polymer overcoat. The metal and polymer provide chemical and optical barriers and a mechanical base for handling the probe. We do not know how long such structures will survive *in vivo*, and the first active chronic implants are now beginning. However, it is likely that the development of encapsulating films for long-term use in biological fluids will become a very important focus for work during the coming decade. There are clearly many challenges here that are not yet understood, especially for applications such as neural prostheses, which have a required operating life that can extend to several decades.

4. *In Vivo* Performance

Over 2500 passive probes such as those in Fig. 1 have now been supplied to physiologists for a wide range of research applications, both acute and chronic. These lithographically defined probes generally record single-unit activity with performance on a par with

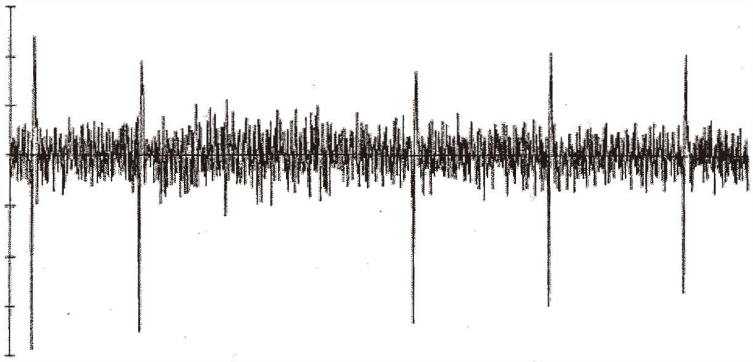

Fig. 4. Single-unit neural activity recorded acutely from guinea pig auditory cortex using a 64-site four-shank probe.

that of metal microelectrodes, which for single-site use are a simple and elegant solution to the recording problem. Figure 4 shows an example of neural recordings from a 64-site passive probe. Chronic probes have recorded single-unit activity for several months; on-chip circuitry allows amplification and multiplexing of the neural signals without significantly degrading baseline noise levels. This is not trivial since the signals of interest extend down below 20 μV and multiplexing involves the use of externally generated 5 V clocks.[11] Tissue reaction to probe presence is minimal so long as the probe floats in the tissue; if it becomes anchored to the skull, then much more reaction is seen. The overall size of the probes is set not by technology but by the mechanical strength needed to penetrate the pia arachnoid over the brain.[20–21]

The most significant problem facing long-term use of such devices for recording is the signal fall-off with time during a chronic implant. This generally occurs after a few weeks or months *in vivo*, although it may occur earlier. The reason for this fall-off is not known with certainty but is thought to be due to encapsulation of the sites by glia. Passing current through the site occasionally can help restore recording ability, possibly due to removal of the glial sheath. Much more needs to be learned about tissue reaction in the immediate vicinity of the probe sites. Work is going forward on the development of various coatings (*e.g.*, proteins) that can enhance, or reduce, the ability of tissue to bond to the probe surface and improve its biocompatibility. Ultimately, this will be an important part of probe packaging.

5. Toward Multifunction Devices

As is well known, the cellular world is primarily chemical in nature. It is important, therefore, to develop micromachined devices that can monitor or control the chemical properties of living tissue in addition to electrically interfacing with the cellular world. In doing so it is important to merge these new functions with the probe process in a way that does not significantly increase the overall process complexity or lower the yield. Figure 5 shows the diagram of an active probe containing integrated microchannels for drug delivery at the cellular level, while Fig. 6 shows two views of an actual probe. These microchannels are buried in the silicon substrate and are fabricated by undercutting a boron-diffused silicon grating structure and then sealing the grating using deposited dielectrics.[22] Channels from 3 μm to over 100 μm in size have been formed with this approach, which requires only one mask in addition to the normal probe process and leaves the probe surface undisturbed for subsequent probe fabrication. The gratings over the channels can be planarized after sealing, but perfect planarization precludes visualization of the flow channels during the remainder of the process.

These structures allow the delivery of chemicals in the 10–100 pL range and have been used for a number of *in vivo* acute studies.[23] We are proceeding to integrate flowmeters within the microchannels and have defined microvalve and pump structures that appear to be compatible with the overall fabrication sequence. This is one of the more exciting applications for microfluidics, and it is clear that it will become the focus of considerable effort. Such devices will open up the field of neuropharmacology in terms of research on

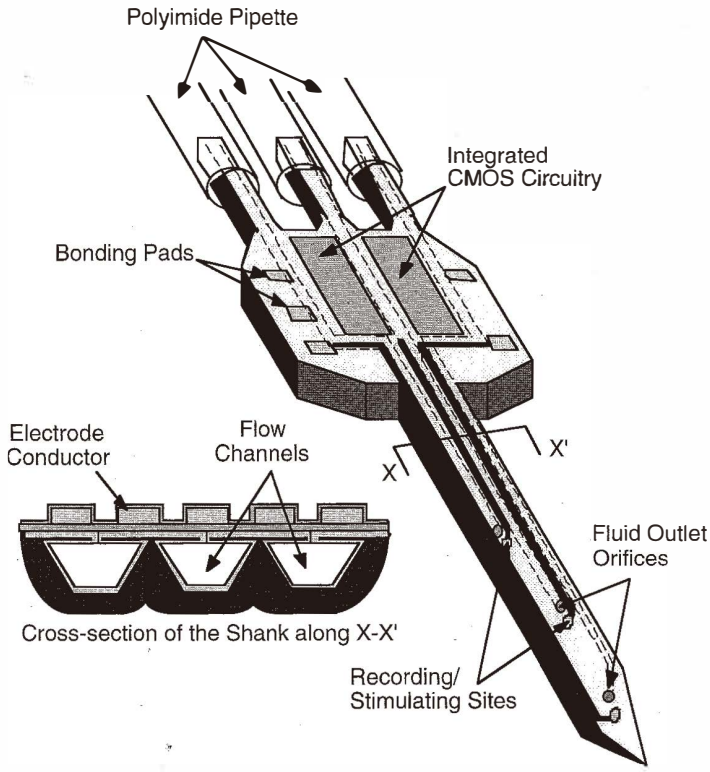

Fig. 5. Diagram of a probe containing microchannels for drug delivery as well as electrical stimulating and recording sites.

the interactions of drugs with living cells and should allow the realization of highly selective surgical and/or therapeutic devices as well.

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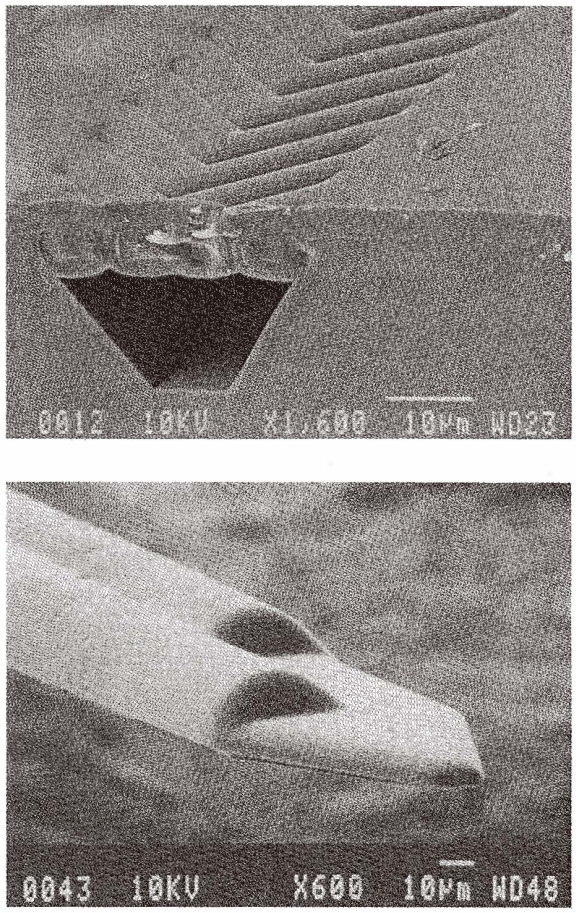

Fig. 6. SEM views of a sealed microchannel in bulk silicon and of the back of a drug delivery probe containing two such channels.

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