REGENERATION MICROELECTRODE ARRAYS FOR DIRECT INTERFACE TO NERVES

Gregory T. A. Kovacs

Center for Integrated Systems, Stanford University Department of Electrical Engineering
CIS 130, Stanford, CA 94305

Abstract

The development and testing of a microelectrode array for implantation in the path of a regenerating nerve is underway. A silicon substrate perforated by an array of holes (*via holes*) is implanted between the deliberately severed ends of a nerve. Axons regenerate through the via holes and thus become spatially fixed with respect to microelectrodes located on the surface of the silicon. Processes were developed for the fabrication of thin-film iridium microelectrodes, micromachined via holes, and silicon nitride passivation layers. All fabrication methods were designed to be compatible with standard CMOS/BiCMOS processes to allow for on-chip signal processing circuits in future designs. This paper provides an overview of these fabrication methods, the *in vivo* testing of passive neural interfaces, and a prototype active circuit version.

Introduction

It is well known that peripheral nerves of vertebrates (including humans) will regenerate if crushed or severed and re-aligned. Axons distal to the injury site undergo a process referred to as *Wallerian degeneration*. Schwann cells in the distal portion of the nerve phagocytize the degenerating axons but remain aligned in "empty" tubes into which regenerating axons can grow [1]. If the proximal portion of the nerve is close enough to the distal portion for regeneration to occur, *growth cones* of the regenerating axons find their way down these tubes. If a thin planar structure, perforated by via holes, is held between the severed ends of a nerve, regenerating tissue will fix the implant in place. A stable electrical mapping between microelectrodes on the substrate and the nerve could be formed by virtue of their fixed spatial relationship. Such devices could potentially be used as bi-directional interfaces between a prosthesis and the nerves of an amputee's limb stump.

The basic concept of regeneration-type neural interfaces is not new, as discussed below. However, implantable devices with on-chip signal processing circuits, required for their practical application, have yet to be realized. On-chip multiplexing schemes are necessary due to the topological limitations imposed by large, tightly-spaced arrays of microelectrodes. In addition, the desire to minimize the number of off-chip connections makes this the most practical approach. Development of such devices has been impeded by the lack of suitably durable and biocompatible materials in addition to fabrication techniques that are compatible with the inclusion of active microcircuits. (Another key impediment is the lack of reliable interconnect technology, which is currently being investigated by several groups.) The history of work in this area is briefly outlined in Table 1.

What appears to be the earliest work (unpublished) was carried out between 1965 and 1967 by Frishkoff, Goldstein, Hambrecht, et al. [2]. They laser drilled 25 μm via holes through 1 cm diameter ceramic "buttons" that were 250 μm thick and implanted the devices in the sciatic nerves of cats. Four electrodes were included on each substrate, and platinum wires were used to make connections to them. After several attempts, it was concluded that neural activity could not be recorded from the arrays. In 1969, Marks [3] also carried out experiments with regeneration-type devices. She demonstrated that bullfrog sciatic nerve axons would regenerate through porous Teflon™ implants placed at the point of transection of the nerve. Unfortunately, the goal of the project, chronic connections to the optic nerve, met with little success [4].

In 1973, Llinás, Nicholson, and Johnson [5] published a paper describing how a silicon-based microelectronic neural interface might someday be implemented. They proposed an array of regeneration electrodes that could be placed between the severed ends of a peripheral nerve. Each electrode would be connected, via multiplexing circuitry, to output amplifiers for recording. Few of the details of such a design were worked out within the scope of their paper, and no devices were implemented.

In the following years, other groups studied regeneration electrodes made on non-semiconductor substrate materials, as indicated in Table 1. In 1978, Matsuo, Yamaguchi, and Esashi [6] reported the fabrication of recording arrays of 200 × 200 μm square via holes etched using a pyrocatechol-based solution in a <100> orientation silicon wafer approximately 200 μm thick. They included MOSFET buffer transistors utilizing a unique "tubular" architecture *within* the via holes. The gates of the tubular MOSFETs lay within and encircled the via holes, with their sources and drains on opposite sides of the wafer. Ten such transistors were fabricated on each substrate. Apparently, no recordings were made due to low signal-to-noise ratios (attributed to high thermal noise levels secondary to large source-drain resistances associated with the "tubular" MOSFET structure) [7].

Year	Investigators	Substrate	Via Hole Fabrication	Biological Results
1965	Frishkoff, et al. [2]	ceramic	laser drilling	no
1969	Marks [3]	Teflon™	porous material used	yes (regeneration)
1973	Llinás, et al. [5]	proposal only	laser drilling proposed	no
1974	Mannard, et al. [8]	epoxy	mechanical drilling	yes (recording)
1977	Loeb, et al. [9]	Parylene™ multilayer	sacrificial metal between layers	no
1978	Matsuo, et al. [6]	silicon	pyrocatechol wet etching	no
1980	Edell [10]	silicon	KOH etching of <110> silicon	yes (recording)
1987	Rosen, et al. [11]	silicon	laser drilling	yes (regeneration)

Table 1: Overview of the historical development of regeneration electrodes.

The first neural signal recordings obtained with a silicon substrate regeneration microelectrode array were reported by Edell in 1980 [10]. Using potassium hydroxide etching of <110> silicon wafers, Edell fabricated large (120 μm × ≈ 1.5 mm) slots in a 140 μm thick substrate. Ten gold microelectrode sites were included on each substrate. Teflon™-coated silver wires were used to make external electrical connections. Neural signal recordings with amplitudes on the order of 150 μV P-P were obtained from such devices implanted in the sciatic nerves of rabbits [10], [12].

Blank silicon substrates with laser-drilled via holes were used by Rosen, et al. [11], as initially proposed (but not implemented) by Llinás, et al. [5], and Edell [10]. Arrays of 50 × 50 via holes with entrance diameters of 50 μm and exit diameters of 25 μm were initially fabricated with a CO_2 laser mounted on a computer-controlled X-Y stage. Subsequent refinements in positioning and the use of a YAG (*yttrium aluminum garnet*) laser, made it possible to drill more cylindrically-shaped holes with entrance and exit diameters of ≈ 8 μm, approximating the diameter of a typical peripheral nerve axon. The blank silicon chips were implanted between the surgically severed ends of rat and primate nerves. This work verified that individual or small groups of axons could regenerate through such small via holes.

Laser drilling is not presently compatible with the fabrication of active microelectronic circuits on the same silicon substrate, due to damage to the surface and substrate [13] and to beam positioning errors [14]. Since, as explained above, the inclusion of on-substrate active signal processing circuitry is a goal of this project, development of the fabrication processes described below was begun.

Passive Microelectrode Arrays

The development of microelectronics processing methods that are not only biocompatible, but also compatible with standard CMOS fabrication processes was undertaken [15]. A plasma-etching technique was developed to allow the fabrication of relatively deep via holes through the substrate (other methods of via hole fabrication such as wet etching, with or without diffused boron etch stops, have also been investigated as alternatives with varying degrees of process compatibility). Using SF_6 and C_2ClF_5 reactant gases, the required aspect ratios (on the order of 10:1) could be achieved.

A lift-off metal deposition technique was employed to permit the use of iridium for the microelectrodes (since iridium is nearly impossible to wet or plasma etch due to its chemical inertness). Iridium was chosen because its properties make it a nearly ideal metal for use when microelectrodes are intended for stimulation [16]. This was desired so that the neural interface could eventually be used to provide sensory feedback from a limb prosthesis.

A plasma enhanced chemical vapor deposition (PECVD) process was used to deposit a biocompatible silicon nitride passivation layer over the devices to insulate the metal conductors from body fluids (and to protect transistors, to be incorporated in future designs, from destruction). Previous work by Kwon [17] indicates that silicon nitride films of this type prevent the penetration of alkali ions that would interfere with MOS transistor operation. The ability to deposit relatively thick layers (up to several microns) of PECVD silicon nitride at low temperatures (250–350° C with present equipment) appears to meet the requirements for compatibility with standard processes.

The above process steps can be combined to fabricate passive microelectrode arrays. Initially, wet thermal SiO_2 is grown on <100> silicon wafers. Typically, iridium metal is deposited by lift-off, using titanium adhesion layers to form a tri-layer of 300Å Ti/3000Å Ir/300Å Ti.[1] The wafers are then coated with 1 μm of PECVD silicon nitride. Contacts through the nitride layer are then plasma etched to expose the microelectrode and input/output pad surfaces (the top Ti layer is then removed). This is followed by the plasma etching of the via holes, the bases of which are opened by mechanically lapping the wafers, using calcined alumina grit. An illustration of the cross-section of a via hole/microelectrode combination is shown in Fig. 1 below.

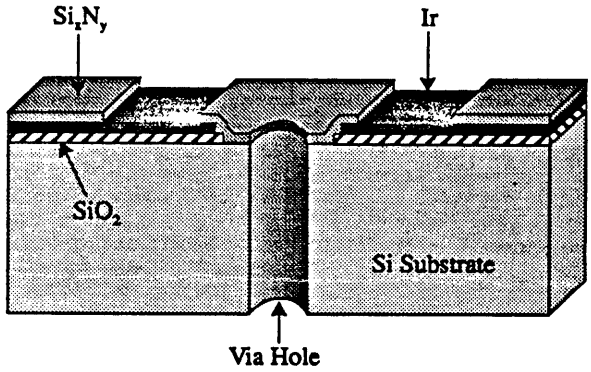

Fig. 1: Diagram of a cross-section of a single microelectrode site. (Not to scale.)

When the above fabrication steps are completed, the individual microelectrode arrays are diced using a standard diamond wafer saw, cleaned, and sorted under an optical microscope. A completed microelectrode array is shown in Fig. 2 below.

During the work described in this paper, *in vitro* measurements of the microelectrode impedance spectra were carried out and indicated impedances of approximately 600 KΩ at 1 KHz for a microelectrode with a 2750 μm^2 surface area. The impedance and phase spectra of an individual microelectrode of this surface area are shown in Fig. 3 and Fig. 4 respectively (measurements were carried out using a 100 mV P-P constant voltage amplitude measurement method described in [18]).

[1]Depending on the design, gold metallization may be deposited to form interconnects and bond-pads using a similar tri-layer approach. For the passive devices described herein, gold interconnects were not used.

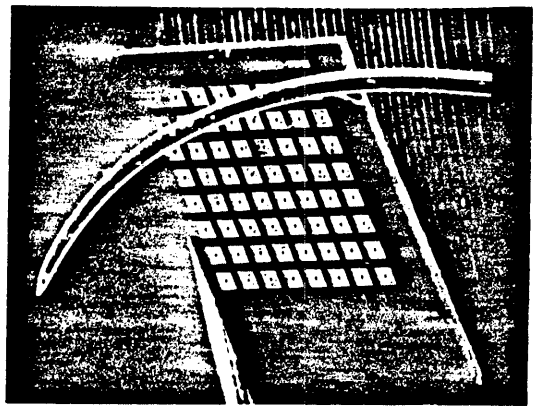

Fig. 2: SEM of one of the passive neural interface designs tested, showing a 10-0 microsurgical needle placed on top of it (magnification = 43X). [SEM by C. W. Storment.]

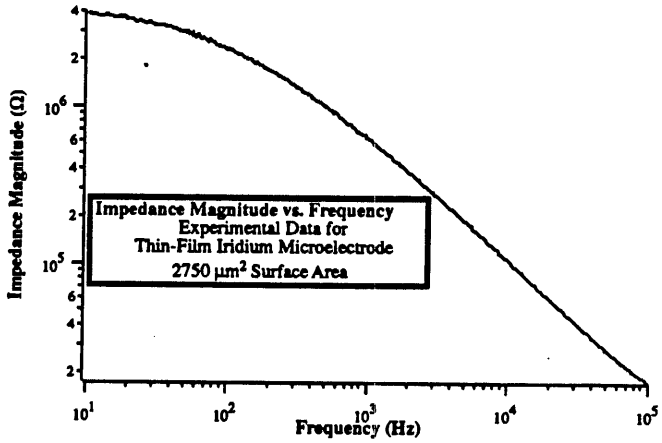

Fig. 3: Plot of impedance magnitude versus frequency for a 2750 μm^2 thin-film iridium microelectrode.

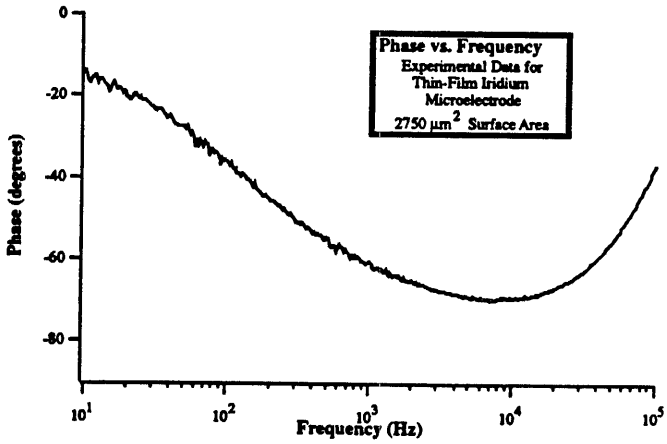

Fig. 4: Plot of phase versus frequency for a 2750 μm^2 thin-film iridium microelectrode.

The completed devices were mounted in pre-cast surgical couplers (donated by Davis & Geck, Inc. Pearl River, N.Y.) and held in place with medical-grade silastic so that the via holes and microelectrodes were located in the bore of the coupler and the signal input/output pads were exposed. Fig. 5 below shows such an assembly prior to implantation.

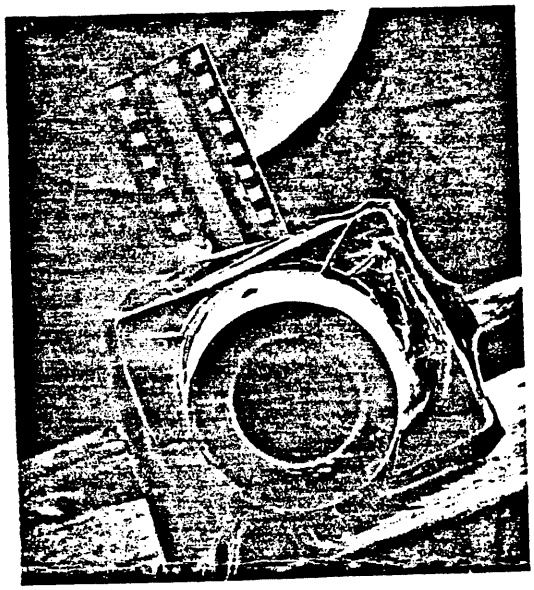

Fig. 5: SEM of a passive neural interface mounted in a surgical coupler (magnification = 16.5X). [SEM by C. W. Storment.]

The devices were implanted in the peroneal nerves of Sprague-Dawley rats using the surgical procedure illustrated in Fig. 6 below. At various post-operative intervals, the implant sites were surgically re-exposed and electrophysiological studies were carried out.

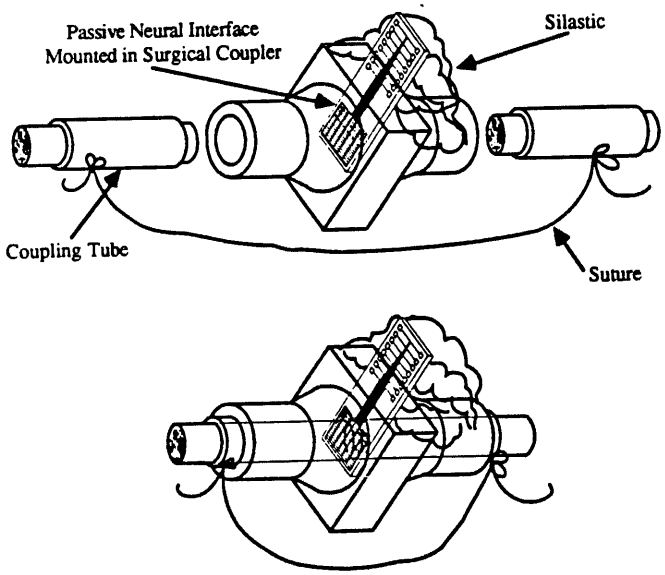

Fig 6: Illustration of the surgical procedure for implantation of neural interface devices in the rat peroneal nerve. [Illustration by K. M. Clark.]

The results of initial implant studies in rats have demonstrated both recording from, and neurostimulation with, the passive neural interfaces at post-operative durations of over one year. After the implant sites were exposed and tissue dissected away from the neural interfaces, electrical connections were made to the signal input/output pads using tungsten probe needles on 3-axis micromanipulators.

Fig. 7 below is a plot of spontaneous action potential signals recorded from a rat peroneal nerve, 377 days from the date of implantation with a passive neural interface. Stimulation of the nerves with the implanted neural interfaces was also demonstrated at comparable durations post-operatively, using extraneural recording electrodes proximal to the implant site. The details of the electrophysiological methods and results are discussed in detail in [19].

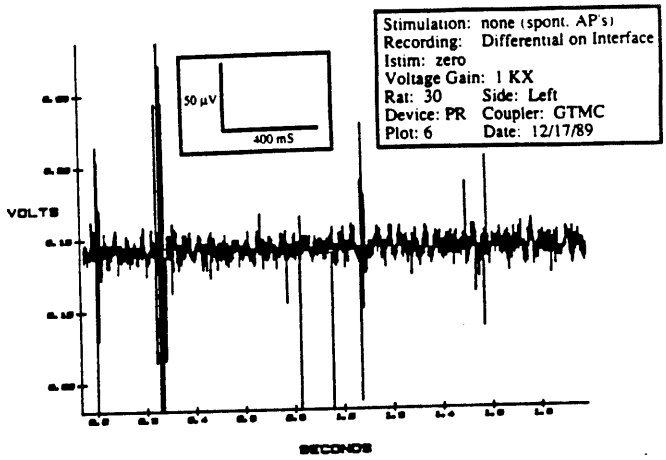

Fig. 7: Plot of spontaneous action potentials recorded from a rat peroneal nerve using a passive neural interface as shown in Fig. 2 above.

Prototype Active Microelectrode Array

In addition to the passive devices described, a non-implantable CMOS prototype active neural interface with much of the requisite circuitry for communication with external electronics has been designed and fabricated [19]. Further work is underway toward the realization of an implantable, active neural interface fabricated using a standard CMOS process with the addition of the process steps described above. A diagram of the sub-circuits of the interface is shown in Fig. 8.

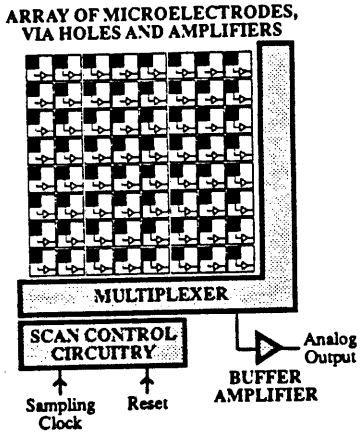

Fig. 8: Diagram illustrating the architecture of the multiplexed, two-dimensional microelectrode array under development.

A simplified prototype version of the active two-dimensional microelectrode array was implemented in 3.0 µm, p-well CMOS using the MOSIS foundry service. The prototype device, shown in Fig. 9, includes a 32 × 32 array of microelectrode sites, multiplexing, sequencing, and timing circuitry. The prototype was fully functional and allowed verification of some of the basic circuit design concepts and also non-contact testing methods required for such devices. Power dissipation was 1.8 mW with a 3 MHz sampling clock.

Liquid-crystal based functional testing was demonstrated. This technique does not have the disadvantages of other methods such as direct probing (impractical and potentially damaging to large arrays) or voltage-contrast electron microscopy (expensive and difficult to apply on a large scale). The device to be tested is coated with a thin film of liquid-crystal material. While operating, the device is viewed under randomly-polarized light (as described in [20]) to verify the operation of circuits using low clock rates. The liquid-crystal material can then be removed with solvents, leaving no detectable residue.

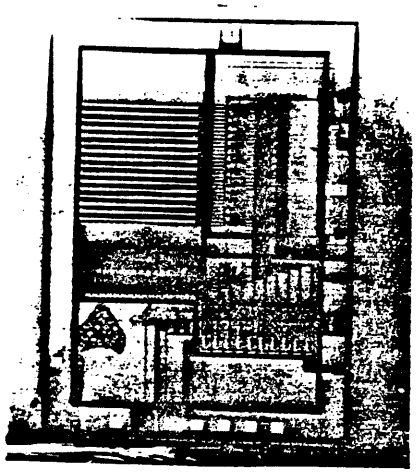

Fig. 9: Prototype active device (magnification = 16.5X). [SEM by C. W. Storment.]

Current Work

Having demonstrated the basic fabrication process and the ability to record from and stimulate peripheral nerves, current work is focused on optimizing the design of the devices from biological and electronic viewpoints. A large-scale study to investigate the optimum design of both the via hole geometry and surgical coupling is underway. Several variations of the devices have been implanted and will be evaluated using both electrophysiological and histological techniques. The *in vivo* spatial selectivity of the microelectrodes is being determined using "second-generation" passive devices fabricated with the basic process and including Teflon™-coated interconnect wires.

Active circuit building-block designs are being completed and fabricated using the Stanford BiCMOS process [21] (as well as MOSIS). Integration of the microelectrode and via hole fabrication steps with the active circuit process is underway. Fig. 10 below illustrates a merged process device cross-section. It is anticipated that this process will be useful for many other applications wherein active circuits and sensors are to be fabricated on the same substrate.

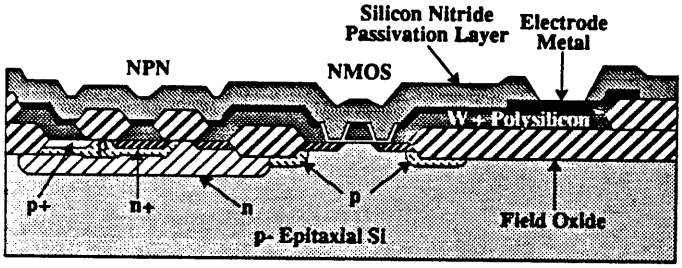

Fig 10: Illustration of a merged BiCMOS/microelectrode fabrication process device cross-section (no via holes are shown).

Discussion

The information obtained from work with first-generation passive and active neural interfaces is being used to design improved devices. Of particular interest is increasing the fraction of the surface area of the neural interface that is open for nerve regeneration. The prototype passive devices, designed to permit only a small number of axons to regenerate through the via holes, were not ideal biologically but did permit the verification of the basic concepts. It is estimated that this (or other) neural interface technology will not be applied clinically for at least a decade. Nonetheless, encouraging technological developments continue in this area.

Many of the details of this work could not be discussed within this paper. A more complete description of the background, theory, work by others, and the present state of this research has been completed [19] and a more detailed summary article is being prepared.

Acknowledgments

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