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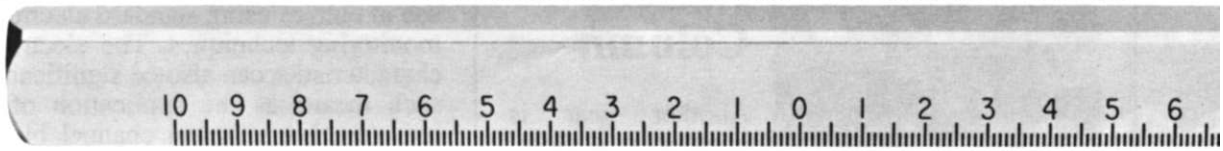

Construction of a Microelectrode Probe Device for Interfacing with the Nervous System

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INTRODUCTION

The formation of an effective and permanent biological interface for communication with the nervous system will be required for the efficient control of prosthetic devices. The multielectrode cable (MEC) device to be described comprises an approach to such an interface. Electronically excitable probe cells are cultured onto an implanta-ble electrode surface which is capable of monitoring extracellular potential. The formation of connections from or to the nervous system with these cultured probe cells could comprise an effective interface, with the electrode contact with probe cells acting as a detector or stimulator depending upon the desired flow of information. This could then allow the utilization of signals from the nervous system to modulate or control the functions which were lost by damage to the nervous system (e.g. muscle movement or prosthetic control). While multielectrode matrices have been used in the past for monitoring cells in culture (Gross et. al, 1977; Israel et. al. 1984; Lucas et. al. 1985) and to some extent in vivo (Bulla-ra et. al. 1979; Gross et. al. 1977), most have design characteristics which make their use with cultured probe cells difficult. The major objectives in the design of the device were implanta-bility, a high density of electrodes, and materials compatible with cell culture and in vivo use.

The primary goals of present study were to establish the ability of the MEC system to detect spontaneous activity of test cells so that they could be monitored to establish viability, and to demonstrate that the expected changes in activity could be recorded following the addition of agents to the medium. For the initial characterization of the operating properties of the MEC, car-diomyocytes were used as short term probe cells in vitro. These cells are easy to harvest, culture and maintain for long periods of time. Like neurons or skeletal muscle, they have documented and identifiable electrophysiological characteris-

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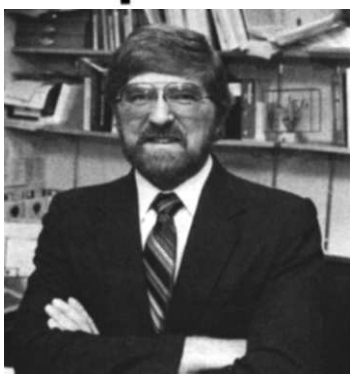

Editor's Column

Another year is coming to a close. By the time this issue of the Carrier is out to you, it will be 1993, and we will be in the process of inaugurating a new president. I went back to last year's New Year edition of the Carrier to see what I wrote about then. We were concerned about the future of the disintegrating Soviet Empire and whether it would be possible for the newly emerging states to survive as free entities or whether the deteriorating living conditions would force a return to totalitarian regimes. Now a year later, the question still remains. However, it does seem more likely that the drive to free trade and democratic governments is stronger. The horrible war between the Serbs and Croats is a grim reminder of the deep seated nationalist and ethnic feelings among the peoples which were formerly held together by force. We must all try to find ways to bridge such chasms and encourage cooperation and understanding between traditional groupings. We are certainly not free of such problems here in the U.S. The riots in Los Angeles and the brewing tensions in New York are evidence that bitter feelings are present and active.

Perhaps as we come into a new year filled with promise and hope, one of the things we could all do is to make a resolution that we will all try to better understand other traditions, and to be more tolerant of beliefs and customs which seem odd or different from that to which we are accustom. After all, diversity in genetics, beliefs, actions and thoughts is what has allowed the human race to be so successful as a species. To attempt to bring homogeneity to human actions and beliefs would not only make it a pretty dull place, but would be to greatly decrease the viability of the species.

Happy New Year

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tics, and we have previous experience with their use in culture using standard electrophysiological monitoring techniques. The electrophysiological characteristics can also be significantly altered by such means as the application of extracellular ions (eg. NA, K), ion channel blocking agents, and Na-K ATPase inhibitors, making them excellent test cells for development of the system hardware.

METHODS

Device Construction

The multielectrode cable assembly designed by this laboratory consists of 10 µ, insulated platinum wires embedded as a square matrix in a polystyrene cylindrical pellet 2-3 mm in diameter and up to 5 mm in length. The cable offers the ability to incorporate numerous electrodes per square surface area. Another advantage is the relative flexibility of the assembly since the electronic hardware are physically located at a distance from the surface containing the test cells. Optical clarity is somewhat compromised, but because the individual electrode surfaces are small relative to the cells and the surrounding support matrix is clear, visualization of the cultured cells can be achieved with optical enhancement.

Polyimide coated platinum (99.999%) wire was purchased from California Fine Wire. Up to 100 segments of wire, each 10 cm long, are assembled together along with another 40 µ diameter wire of similar composition which serves as a ground plane. Individual wires are threaded through two aligned nylon screens with approximately 50 µ mesh size (Small Parts Inc., Miami, FL) which have been attached to a cardboard frame. The wires are threaded through consecutive spaces in a square or circular array (Fig. 1A). This allows the bundle of wires at one end to be configured in a precise X-Y matrix. A sleeve of silastic tubing is fitted around the matrix end of the wire bundle and secured in place with silicon adhesive. For the formation of the pellet, culture dish polystyrene (Falcon Plastics) is dissolved in HPLC grade chloroform (approximately 1:1 by weight) at room temperature. The solution is injected into the interior of the tube, filling the spaces around the wire bundle (Fig. IB). The support frame which houses the wire bundle is placed at -20°C for one week, then transferred to a 4°C refrigerator for 3-4 days and finally allowed to sit at room temperature for one week before trimming the surface.

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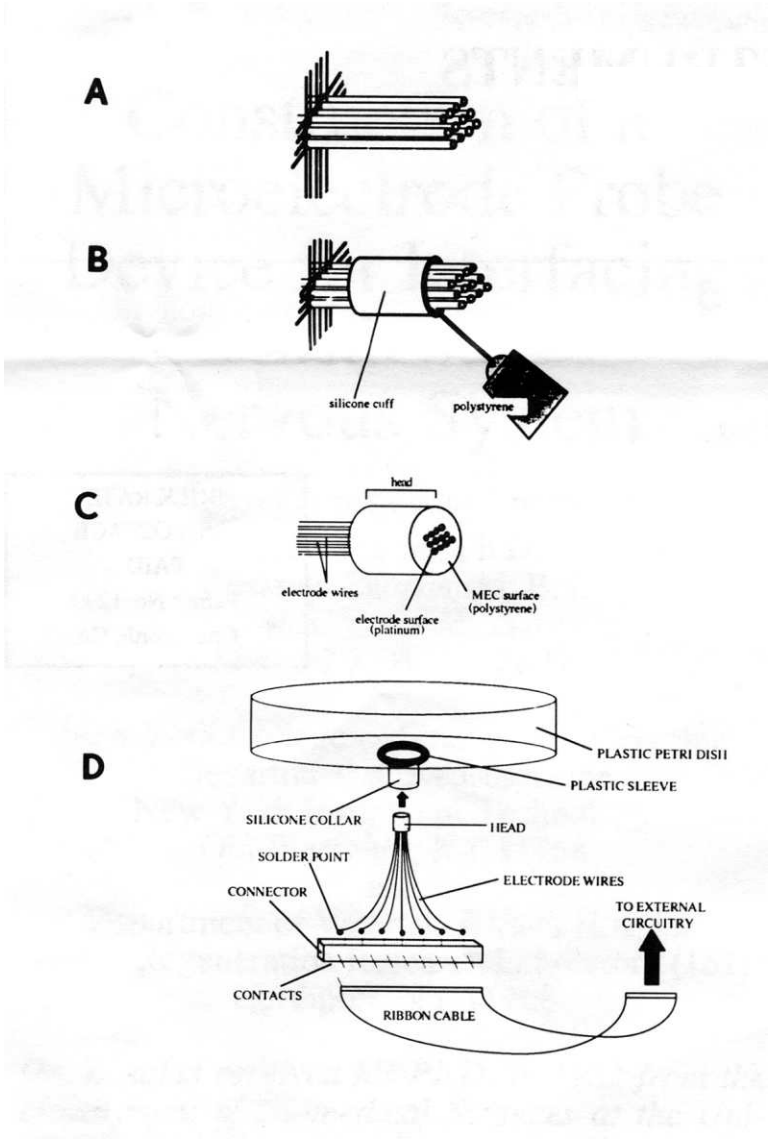

Figure 1. Schematic steps in the construction of the MEC and its use for in vitro recording. (A) Al-lignment of wires through mesh screening. (B) Application of a Silastic cuff and polystyrene. (C) Trimmed MEC surface. (D) Setup for recording from cell culture.

This allows the solvent to evaporate slowly so that bubbles of vaporized solvent are not formed in the pellet. The hardening at room temperature improves trimming characteristics. The matrix end of the cable (which will serve as the MEC surface or recording plane) is then cut on a microtome or can be trimmed by hand with a razor blade under a dissecting microscope. The result is a surface with uniformly flat metallic electrode ends which form a recording plane embedded in polystyrene with wire separation of 25 to 50 microns (Fig. 1C). The free ends of the wires are stripped of insulation with wire stripper (PT-5-ML, London Chemical Co., Inc. Bensenville, IL) to a length of 5 mm and soldered to the pin terminals of a ribbon cable connector under a dissecting microscope (Fig. ID). This provides a removable connector for interfacing with operational amplifier buffers. The electrode wires can then be led to high input impedance non-inverting unity-gain buffers. Terminals are then examined for conduction with an ohm meter with the MEC recording surface immersed in a saturated saline solution. Insulating material is painted on the stripped ends of the wires adjacent to the connector to avoid short circuiting of the stripped sections of the wires. Over 95% of electrode terminals are functional by these methods.

For cell culture on the electrode, the surface can be dipped in collagen, polylysine or other extracellular matrix solutions (usually at a 0.1% concentration) to promote cell adhesion. A 0.20 mm thick silastic band which is 5 mm long is placed tightly around the MEC surface. The assembly will then be fitted through another polystyrene tube secured into the base of a 35mm plastic culture dish (Falcon), thereby securing the MEC surface to the inside bottom of the dish (Fig. ID).

Isolation of Probe Test Cells

Cardiomyocytes were isolated in calcium free medium from young adult rats by the method of Rossner (1991). Approximately 35-40% of myo-cytes survive reexposure to calcium by this method. Cell density was diluted to approximately 100,000 cells/lOOul and a volume of 15|il placed on each MEC surface for recording.

MEC Recording from Probe Cells

The MEC surface with cultured cells was placed into an upright microscope stage designed to securely hold the culture dish and protruding wires. The MEC surface was epi-illuminated with a halogen fiberoptic lamp and a helium-neon laser placed at right angles to each other. The MEC surface was visualized with a long working distance objective (16X, Zeiss) and 20X oculars in the microscope equipped with a trinoc-ular head and a vidicon camera. The use of this optical system for in vitro studies allowed the recording of a cell on a particular MEC electrode

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in a unambiguous fashion by simultaneously recording from the same cell using standard extracellular electrophysiological techniques. Briefly, the video cameras allowed the micromanipula-tion of a glass micropipette into close apposition to the test cell of interest so that spontaneous bursting activity could be recorded from the identified cell.

The relevant external circuitry included the recording channels, where the low impedance signals from the buffer circuit were led into an adjustable high-gain amplifier (Grass P15 or P16). The signals from each channel were monitored and recorded simultaneously by multiplexing each output channel at 20KHz, thus maintaining good signal fidelity while not exceeding the frequency response of the recording device. All of the electrophysiological determinations were performed in a Faraday cage, constructed on a vibration-free table.

Monitoring of spontaneous spike activity was initiated 1 hour after inoculation to establish active electrodes, baseline values, and sources of artifact. Since the monolayer cell cultures produce a great variety of spontaneous activity including tonic and phase firing, it is useful to try to detect random or rhythmic events. The active leads were identified first by channel selection using a data acquisition/data analysis system developed especially for this purpose. Monitoring was performed continuously for each MEC channel.

RESULTS

After the reintroduction of calcium to the medium (Rossner 1991), standard extracellular signals could be obtained from cardiomyocytes through the active MEC channels. Both spontaneous activity (Fig. 2A) and stimulus evoked responses (Fig. 2B) could be obtained. Where myocytes could be observed lying directly over an electrode (Fig. 2A), signal amplitudes of 0.9 millivolts with an action potential duration of 1.0 millisecond (representing 75% of complete repo-larization, Fig. 2A) were observed. These characteristic are similar to those obtained by previous investigators (Israel *et al.* 1984) after the addition of epinepherine 925 µg/ml). Killing of the cell by the application of a strong direct current abolished all responses.

The identity of the cell could be verified through the simultaneous stimulation of the cell by a visually guided external electrode as described above. While monitoring the MEC and conventional electrode channels, square-wave stimuli were applied via a fine stimulating micro-pipette. Stimulus-isolation circuits were used to minimize shock artifacts. Evoked as well as spontaneous responses from the test cells could be compared between conventional and MEC recorded data. Figure 2D shows the responses of MEC and conventional electrodes recording from the same cell. This type of dual observation also made it possible to directly demonstrate that direct contact with the metal surface is not necessary to allow monitoring. Electrodes with a separation of 10-20u from the stimulated myocyte could still yield reliable and reproducible results.

SUMMARY AND DISCUSSION

In vitro testing of the MEC system using cardiomyocytes has demonstrated that the device is capable of reliably detecting normal extracellular electrophysiological signals from cells cultured on its surface. There are limiting factors for obtaining successful recording from a particular electrode. The cultured test cells must make contact with or be within recording range of the individual electrodes of the MEC. Thus, when cells are cultured on the MEC surface, the number of actively recording electrodes are smaller than the actual number of electrode surfaces. This is because in conventional cultures which are inoculated at optimal densities, intercellular distances are about 15 (I. Thus, inoculation density is the most important variable in establishing the proportion of electrode to probe cell adhesions.

While these experiments demonstrate that the in vitro properties of the MEC are consistent with the detection of physiological signals in vivo, the transition will involve new problems including probe cell stability on the electrode, and problems associated with implantability including tissue reactions, and the formation of neural connections with probe cells. This may dramatically affect the proportion of probe cells yielding detectable signals and the magnitude of the signals themselves.

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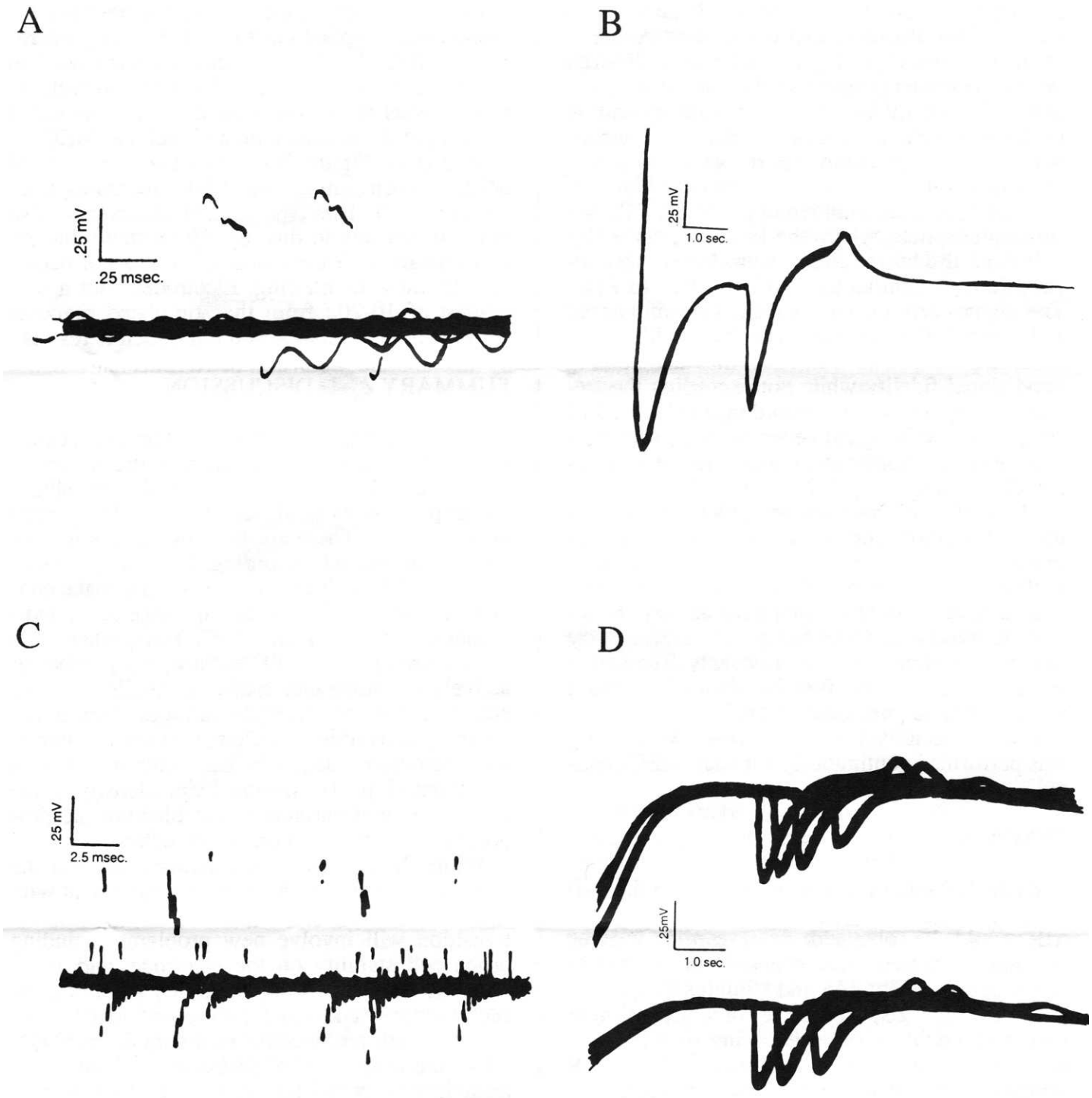

Figure 2. Oscilloscope tracings from cardiac myocytes on the MEC surface. (A) Tracing of two similar overlapping episodes of spontaneous activity from a cell overlying an electrode. (B) Evoked potential after a 4.0 volt, 3.5 msec, stimulus. (C) Epinepherine (25 μg/ml) stimulation of spontaneous activity. (D) Evoked potentials (IIV, 8.5 msec, stimulus) from the same cell simultaneously recorded through the MEC (electrode upper trace) or a conventional electrode (lower traces).

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