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Review

A critical review of cell culture strategies for modelling intracortical brain implant material reactions

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ABSTRACT

The capacity to predict *in vivo* responses to medical devices in humans currently relies greatly on implantation in animal models. Researchers have been striving to develop *in vitro* techniques that can overcome the limitations associated with *in vivo* approaches. This review focuses on a critical analysis of the major *in vitro* strategies being utilized in laboratories around the world to improve understanding of the biological performance of intracortical, brain-implanted microdevices. Of particular interest to the current review are *in vitro* models for studying cell responses to penetrating intracortical devices and their materials, such as electrode arrays used for brain computer interface (BCI) and deep brain stimulation electrode probes implanted through the cortex. A background on the neural interface challenge is presented, followed by discussion of relevant *in vitro* culture strategies and their advantages and disadvantages. Future development of 2D culture models that exhibit developmental changes capable of mimicking normal, postnatal development will form the basis for more complex accurate predictive models in the future. Although not within the scope of this review, innovations in 3D scaffold technologies and microfluidic constructs will further improve the utility of *in vitro* approaches.

1. Introduction

The control of the symptoms of Parkinson's disease with deep brain stimulators (DBS) [1] (Fig. 1A) and the control of prosthetic limbs with BCIs [2] utilizing macro scale electrodes have shown significant success for interfacing directly with the CNS. The success of macro electrode devices holds great promise for intracortical microdevice BCIs. However, recent research has sought to develop devices with cell-level selectivity for either recording or stimulating applications. High acuity vision prostheses (stimulating application) and fine control prosthetic limbs (recording application) both require microelectrodes with high spatial resolution. To achieve the high spatial resolution electrode size must be decreased substantially beyond current commercial technologies. The issues associated with scar tissue encapsulation and poor neural cell interactions are thus applicable to both recording and stimulation devices. Successful resolution of these issues will come from the development and design of microdevices and materials which have appropriate electrical properties (see Cogan [3] and Merrill et al. [4], for in depth reviews on safe electrical stimulation parameters for excitable tissues), combined with in depth understanding of the intracortical tissue reactions. While research has sought to understand the limiting interactions between such devices and cortical tissues, current animal models have been unable to provide definitive answers or yield eloquent solutions to improving chronic implant safety and performance. The focus of this review is to discuss how *in vitro* cell culture can be utilized to model and provide insights into the microdevice/central nervous system (CNS) tissue integration issues.

The development of biomedical devices designed to function in concert with the mammalian nervous system have the potential to enhance the quality of life of many individuals. Such devices include intracortical penetrating electrodes that are capable of interfacing with small populations of neurons; these devices often require a high level of spatial resolution (Fig. 1C and D). Other devices requiring the ability to record or stimulate large, diffuse populations of neurons include those positioned on the surface or outside the brain, such as micro electrocorticography (micro-EcOG) arrays (Fig. 1B). The way in which these devices interact and hence communicate with neural tissue is critical to their long-term

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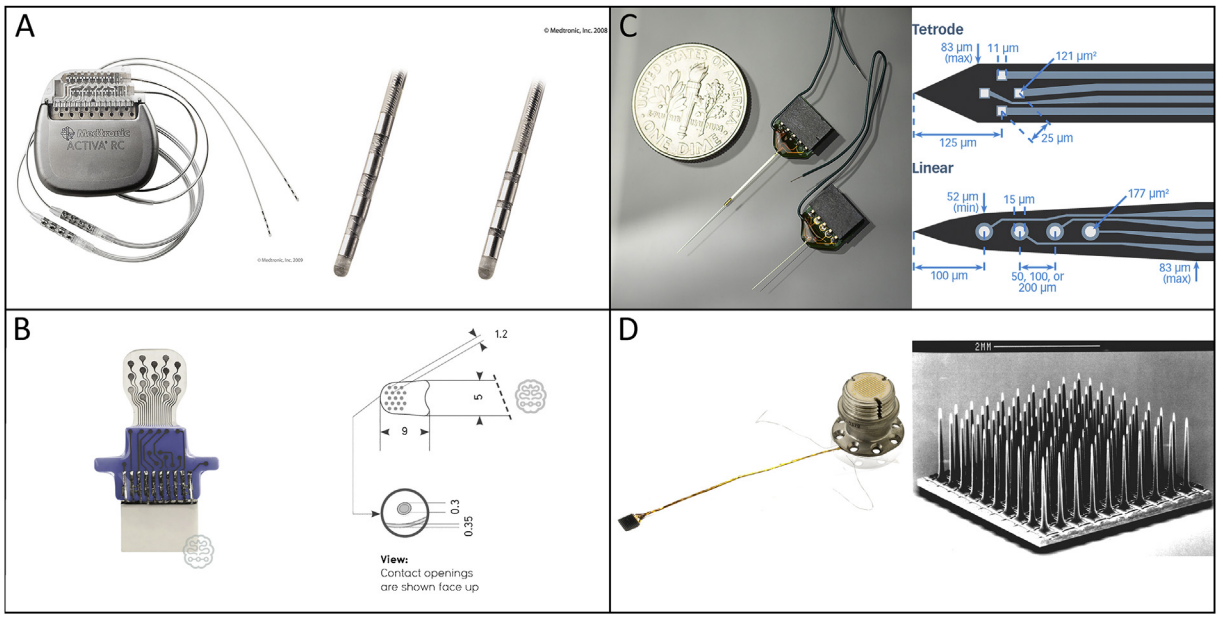

Fig. 1. Types of electrode arrays for direct interfacing with populations of CNS neurons A) Medtronics deep brain stimulator, with two tripolar electrode array variants on the right side with electrode lengths of 1500 µm used for stimulating large populations of neurons. https://professional.medtronic.com/ B) Surface (non-penetrating) electrocorticography (EcOG) BCI interface array for interfacing with relatively large populations of neurons micro arrays from CorTec (units mm) http://cortec-neuro.com/en/. (C & D) Both are intracortical penetrating electrodes designed for both recording and stimulating small populations of neurons. C) Variant of a Michigan array from NeuroNexus http://neuronexus.com/products/neural-probes. B) Utah array from Blackrock microsystems http://www.blackrockmicro.com/.

function. While some devices are primarily used for recording from neurons, others seek to stimulate neural activity. Independent of application, the ability to pass charge between the synthetic device and the neural cells is largely dependent on the reaction of the tissue to the presence of the device. Despite decades of research, the variability and control of these cell mediated responses is still not fully understood and has been shown to change over time. *In vitro* approaches are commonly used to assess device and material compatibility with cells, but have the capacity to help researchers understand critical cell responses that may affect long-term performance of cortical implant devices [5].

Biomedical devices for neural interface applications include those that restore sensory or motor function, and those that enhance regeneration of neural tissues. Device types include restorative prostheses such as bionic eye, cochlear ear implants, neurally driven robotic limb prostheses and deep brain stimulators. Regenerative scaffold implants are being utilized to promote healing in the damaged central or peripheral nervous system (CNS or PNS). Future microdevice technologies have been proposed that incorporate elements of both restorative and regenerative implants, combining both electrically driven interactions and tissue engineered interfaces for biointegration. This emerging concept aims to enhance device integration and function over chronic implant timeframes, with the 'living electrode' being one such example [6–8]. The development of these materials and devices require intensive testing at a number of stages to assess toxicity, material stability in the biological milieu, ability of the material to integrate and support the normal function of surrounding tissues and ultimately perform the desired function over periods of years [9] (For in depth reviews of current and future BCI devices see Lebedev [10] and Lebedev & Nicolelis [11]).

A core issue that currently hinders the widespread application of penetrating intracortical electrodes in biomedical microdevices is the high rate of failure in chronic settings [12]. Electrode failure can be defined as any event which results in signal-to-noise ratio (SNR) that renders the signal meaningless, an impedance value that breaches the safe charge injection limits of the electrode material [13–15], or any event that disables the electrode. There are numerous reports indicating long-term *in vivo* functioning of implanted neural electrodes, however often 40–50% of all electrode sites in an array are non-functional or their function degrades to a point where they fail to either stimulate or record electrical signals in the surrounding neural tissue [16,17]. Additionally, there have been reports of entire electrode arrays failing within a few months of implantation [18]. The exact causes of electrode or device failures in the current literature are often not reported, however the majority of the literature points towards biological events as the principle cause of progressive electrode failure [9,12,16,17].

The factors leading to electrode failure *in vivo* are multifaceted and complex [12], as they can result from material failure (e.g. electrode delamination), mechanical failure (e.g. fracture of electrode shank), biological reactions or any combination of these [17]. The biological response to device implantation and its chronic presence results in a complex array of reactions that can contribute to device failure in time frames from a few weeks to years [16–18]. Studying the causes and the sources of these reactions *in vivo* is challenging for a number of reasons including myriad challenges associated with methods of tissue assessment [19], animal welfare, limited experimental control, and cost of experiments [20]. Investigation of the biological reactions has the potential to provide insight into this failure mechanism however data from both *in vivo* and *in vitro* research currently do not provide clear answers.

A significant *in vivo* challenge is the method of assessment and the time points at which the device-tissue reaction is assessed post implantation. The collection, processing and imaging of histological samples from *in vivo* experiments have significant limitations, as discussed in Woolley et al. [19]. Few *in vivo* studies have successfully followed the cellular/tissue reactions around cortical implants over time, and each of these studies has significant limitations. Kozai et al. [21], utilized two-photon microscopy to assess microglia and vasculature responses to device implantation but did not find a correlation between tissue reaction and changes in electrode