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Nanostructuration strategies to enhance microelectrode array (MEA) performance for neuronal recording and stimulation

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ABSTRACT

Microelectrode arrays (MEAs) are widely used tools for recording and stimulating extracellular neuronal activity. Major limitations when decreasing electrode size in dense arrays are increased noise level and low charge injection capability. Nanostructuration of the electrode sites on MEAs presents an efficient way to overcome these problems by decreasing the impedance of the electrode/solution interface. Here, we review different techniques used to achieve this goal including template assisted electrodeposition for generating macro- and mesoporous films, immobilization of carbon nanotubes (CNTs) and deposition of conducting polymers onto microelectrodes. When tested during in vitro and in vivo measurements, nanostructured MEAs display improved sensitivity during recording of neuronal activity together with a higher efficiency in the stimulation process compared to conventional microelectrodes.

1. Introduction

Almost 40 years ago the first microelectrode array (MEA) was designed to record electrical signals from cultured cells (Robinson, 1968; Thomas et al., 1972). Since this time a lot of progress has been achieved in the domain, especially during the last 15 years due to the commercial availability of MEA systems and affordable computing power (Anderson et al., 1989; Gross et al., 1982). A variety of different MEA layouts were fabricated using lithographic techniques, reflecting a wide spectrum of in vitro or in vivo applications (Bai et al., 2000; Egert et al., 1998; Maynard et al., 1997). MEAs are used for various purposes including pharmacological studies in dissociated neuronal networks, different stimulation implants (cochlear, retina, cortical, spinal) and fundamental research aiming at better understanding the functioning of neuronal networks. Compared to neurobiological measurements performed with single electrodes (e.g. tungsten or glass capillary electrodes) MEAs offer the advantage that information from electrogenic cells can simultaneously be analyzed in different regions of the same tissue over long periods of time (Csicsvari et al., 2003). The acquired data can be used to map the spatio-temporal dynamics of the activity in neuronal networks in order to study interactions between the different cell populations (Heck, 1995). Further evolution of MEA measurement technology requires maximization of available information density. A recent trend in MEA research consists in working with microelectrodes of reduced size. In this way recording and stimulation can be confined to small populations up to single neurons preventing the interference with neighboring cells. Hence the number of electrode sites on one array can be increased leading to high density microelectrode or microtransistor arrays with very high spatial resolution (Berdondini et al., 2009; Charvet et al., 2010; Frey et al., 2009; Hutzler et al., 2006). However decreasing the geometric area of the microelectrodes results in an increase of electrode impedance and consequently higher electronic noise during recording of neuronal signals. Equally the charge that can be safely injected through an electrode in order to stimulate the surrounding tissue is reduced (Cogan, 2008). Those factors present serious drawbacks for using microelectrodes of small surface area on MEAs and current work should now focus on strategies to overcome these limitations. Generally this can be achieved by increasing the effective surface area of an electrode while maintaining its small overall geometry. This presents a widely used approach to improve the performance of electrodes in application fields including analytical chemistry or energy conversion systems. Different techniques exist that allow increasing the active surface area of electrodes by creating porous, nanostructured surface morphologies. Among these approaches some have been applied to MEAs in order to enhance their performance at the interface with the solution containing cultured cells. The objective of this contribution consists in presenting different

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nanofabrication techniques that have been successfully adapted to MEAs and to point out their utility for improved neuronal recording and stimulation processes.

2. Interfacial behavior of microelectrodes in neural recording or stimulation

In order to understand which parameters are of a special interest in neurobiological recording and stimulation with MEAs, a brief description of both processes is given here with a special emphasis on the processes occuring at the interface between the electrode and the solution. When an electrogenic tissue is immersed in physiological solution in close proximity to the electrodes of MEAs, extracellular signals that may originate from different cells can simultaneously be recorded at the electrode. Electrical activity of the cells causes a current to flow around its signal source in the extracellular medium. This current corresponds to a voltage gradient present in the extracellular fluid which is a function of the number, spatial distribution and orientation of active cells. The voltage finally is recorded at the microelectrode site as a function of time with respect to a reference electrode that is immersed into the physiological solution. The electric signal is then further amplified and filtered before signal post-processing steps can be performed with specially adapted software. Although several parameters in this acquisition chain may be important, a crucial factor directly affecting the quality of recorded signals is the interface between the electrode and the extracellular fluid. A small area in contact with the solution results in high electrode interfacial impedance and consequently a high thermal noise which can be expressed by (Gesteland et al., 1959; Heuschkel et al., 2002; Johnson, 1928):

$$N_{th} = \sqrt{4K_B T \int Re(Z)df}$$

In this equation N_{th} corresponds to the rms value of the thermal noise (V), K_B is the Boltzmann constant, T is the temperature (K), $Re(Z)$ is the real part of the microelectrode interfacial impedance (Ω) and f is the frequency (Hz). Low sensitivity in the recording process due to high noise therefore especially presents a problem for small microelectrodes on MEAs. Here, a significant decrease in impedance has to be obtained in order to reach signal-to-noise ratios (SNRs) that allow detection of neuronal signals. The stimulation process of electrogenic tissues relies on an inversion of the above mentioned events. Now current pulses that are applied to the electrode induce a change of the distribution of ionic species in solution so that a potential field is established throughout the electrolyte, which may eventually activate cells (see Joucla and Yvert in this issue). An injection of current into an electrode generally may result in different forms of charge transfer, a capacitive and eventually also a faradaic charge transfer. As long as the current amplitude and hence the potential at the electrode does not exceed a certain value only redistribution of charge carriers happens, resulting in charging of the double layer at the interface between electrode and electrolyte. Inversing the polarity of the applied current leads also to a reversal of the charge distribution at the interface. This capacitive charge transfer is reversible and no electron transfer occurs between the electrode and the solution, which is a characteristic found in electrical capacitors. In contrast, a faradaic charge transfer involves electron transfer from the electrode to the electrolyte and vice versa due to reduction or oxidation reactions occuring at the electrode surface. Faradaic reactions can happen when the current values injected into an electrode are sufficiently high to generate an overpotential that is needed for certain redox reactions to take place. Principal faradaic reactions at metal surfaces in aqueous solutions include the reversible formation and reduction of oxide layers, but also oxidation or reduction of water as well as corrosion reactions (Kovacs, 1994). Therefore currents injected into a microelectrode should not exceed a certain limit in order to avoid irreversible faradaic reactions at the electrode surface. Besides damaging of the electrode by corrosion, these reactions may be responsible for cell death due to accumulation of reaction products in solution, creating a toxic environment for cells. The maximum charge that can be injected "safely" through a microelectrode depends on several factors including the material used for the electrode, the electrolyte, stimulation parameters as well as the size and shape of the electrode (Merrill et al., 2005). Obviously microelectrodes of small overall dimensions may safely inject less charge than macroelectrodes. Thus sufficient charge injection into the electrode often presents the limiting factor for efficient stimulation of the surrounding tissue with MEAs. Increasing the effective surface area of microelectrodes therefore not only leads to a decrease of impedance and thermal noise of the electrode, but also allows to inject higher charges, necessary for an efficient and reliable stimulation process of electrogenic tissue. Besides the tissue damage that is caused by electrochemical reactions at the electrode, also a second mechanism referred to as the mass action theory has been described. Here, the tissue is overstimulated due to many neurons firing simultaneously or over prolonged times which causes the local environment, e.g. ionic, oxygen or glucose concentrations to change (Merrill et al., 2005). The domain of safe stimulation will depend on the injected charge density and the charge injected per phase for different types of tissue (Haberler et al., 2000; McCreery et al., 1990). In deep brain stimulations the limit for safe stimulation conditions was found to be 30 $\mu C/cm^2$ for an injected charge of 2 μC per phase (Kuncel and Grill, 2004).

3. Electrode materials in MEAs

A short overview on electrode materials suitable for the use in MEAs is given in this chapter. Well documented reviews on stimulation of electrogenic tissue and the electrode materials employed for this purpose are available in the literature (Cogan, 2008; Merrill et al., 2005; Robblee and Rose, 1990). Principally some basic criteria have to be met for an electrode material in MEAs. First of all the proximity of living tissue to the electrode material absolutely demands biocompatibility to avoid a necrotic cell response as observed for example for cells that were cultured on silver. Ideally, a cell attachment to the material allows a stable junction to be formed between electrode and tissue, a factor which is of importance especially in long term in vitro and in vivo measurements. Electrode materials further should offer sufficient mechanical strength combined with stable electrical properties guaranteeing reliable long term performance of the MEA. High charge injection capacity per surface area is a key parameter for electrode materials used for efficient stimulation of electrogenic tissue. A way of achieving safe stimulation conditions presents the use of capacitor electrodes (Guyton and Terry Hambrecht, 1974; Guyton and Hambrecht, 1973). Here the underlying metal electrode is separated from the electrolyte by a dielectric material (TiN, Ta_2O_5) allowing charge to be injected into the tissue strictly by capacitive action (Hämmerle et al., 2002; Janders et al., 1996; Merrill et al., 2005; Norlin et al., 2005; Rose et al., 1985; Walter and Heimann, 2000). This prevents potentially destructive faradaic reactions at the electrode surface. The maximum charge however that can be injected into a smooth electrode just by capacitive coupling is about 20–30 $\mu C/cm^2$ (Rose et al., 1985). Considering a typical stimulation protocol of 50 μA for 200 μs pulses per phase (Jackson et al., 2006), a capacitor electrode reaches this maximal injectable charge density when its diameter exceeds 220 μm which is much too big to enable stimulation in a very confined area. To be able to inject higher currents, materials showing reversible faradaic reactions