Published in final edited form as:

J Neurosci Methods. 2011 June 15; 198(2): 222–229. doi:10.1016/j.jneumeth.2011.04.004.

Ceramic-based microelectrode arrays: recording surface characteristics and topographical analysis

Pooja M. Talauliker^{a,b,c,1}, David A. Price^{a,b,c}, Jason J. Burmeister^b, Silpa Nagari^d, Jorge E. Quintero^{a,b,c}, Francois Pomerleau^{a,b,c}, Peter Huettl^{a,b,c}, J. Todd Hastings^d, and Greg A. Gerhardt^{a,b,c}

Pooja M. Talauliker: pooja.talauliker@uky.edu; David A. Price: david.a.price@uky.edu; Jason J. Burmeister: jasonburmeister@yahoo.com; Silpa Nagari: snagari@cadence.com; Francois Pomerleau: francois.pomerleau@uky.edu; Peter Huettl: peter.huettl@uky.edu; J. Todd Hastings: todd.hastings@uky.edu; Greg A. Gerhardt: gregg@uky.edu

^a Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY, USA, 40536

^b Center for Microelectrode Technology, University of Kentucky, Lexington, KY, USA, 40536

^c Morris K. Udall Parkinson's Disease Research Center of Excellence, University of Kentucky, Lexington, KY, USA, 40536

^d Department of Electrical and Computer Engineering, Center for Nanoscale Engineering, University of Kentucky, Lexington, KY, USA, 40536

Abstract

Amperometric measurements using microelectrode arrays (MEAs) provide spatially and temporally resolved measures of neuromolecules in the central nervous system of rats, mice and non-human primates. Multi-site MEAs can be mass fabricated on ceramic (Al_2O_3) substrate using photolithographic methods, imparting a high level of precision and reproducibility in a rigid but durable recording device. Although the functional capabilities of MEAs have been previously documented for both anesthetized and freely-moving paradigms, the performance enabling intrinsic physical properties of the MEA device have not heretofore been presented. In these studies, spectral analysis confirmed that the MEA recording sites were primarily composed of elemental platinum (Pt^0). In keeping with the precision of the photolithographic process, scanning electron microscopy revealed that the Pt recording sites have unique microwell geometries post-fabrication. Atomic force microscopy demonstrated that the recording surfaces have nanoscale irregularities in the form of elevations and depressions, which contribute to increased current per unit area that exceeds previously reported microelectrode designs. The ceramic substrate on the back face of the MEA was characterized by low nanoscale texture and the ceramic sides consisted of an extended network of ridges and cavities. Thus, individual recording sites have a unique Pt^0 composition and surface profile that has not been previously observed for Pt-based microelectrodes. These features likely impact the physical chemistry of the device, which may influence adhesion of biological molecules and tissue as well as electrochemical recording

© 2011 Elsevier B.V. All rights reserved.

Corresponding Author: David A. Price, Ph.D., University of Kentucky, Department of Anatomy and Neurobiology, Center for Microelectrode Technology, Morris K. Udall Parkinson's Disease Research Center of Excellence, 306 Davis Mills Bldg. 800 Rose Street, Lexington, KY 40536-0098, P: 1-859-323-6092, F: 1-859-257-5310, David.A.Price@uky.edu.

¹Current affiliation: Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY, USA, 40536

Disclosure Statement

Greg A. Gerhardt is owner of Quanteon, LLC (Nicholasville, KY) that makes the FAST-16 recording system used with MEAs.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

performance post-implantation. This study is a necessary step towards understanding and extending the performance abilities of MEAs *in vivo*.

Keywords

biocompatibility; ceramic implant; central nervous system; electrochemistry; neural device; platinum electrode

1. Introduction

Our laboratory has pioneered the development and implementation of ceramic-based microelectrode array (MEA) technology coupled with amperometry for the real-time monitoring of central nervous system (CNS) neurotransmitters. MEAs containing 4–16 thin film platinum (Pt) recording sites are routinely fabricated using photolithographic methods, which impart a high level of reproducibility while permitting the arrangement of multiple recording sites in various geometric configurations in a rigid but durable recording device (Burmeister and Gerhardt, 2006; Hascup et al., 2007).

Historically, Pt has been used for decades as an amperometric recording surface (both planar and wire-based designs) because of its long-lasting biocompatibility and high catalytic properties for hydrogen peroxide (H_2O_2) (Gerhardt and Burmeister, 2000). However, a review of the current scientific literature revealed that the recording performance per unit area varies for the detection of H_2O_2 at different Pt-based electrodes with MEA performance rivaling or exceeding that of other electrode subtypes (Table 1). An important aspect of MEA technology is the ability to perform site-specific electrode modifications of the uniform, spatially defined recording sites (Burmeister and Gerhardt, 2001). Exclusion layers are commonly employed to reduce electrode responsiveness to common CNS interferents such as ascorbic acid (Burmeister et al., 2000; Burmeister et al., 2008). In addition, enzymes can be directly immobilized on recording surfaces to convert a non-electroactive neurochemical (*e.g.* glutamate) into a reporter molecule (*e.g.* H_2O_2), which can be quantified upon contact with the electrode surface (Burmeister et al., 2000; Burmeister and Gerhardt, 2001). Importantly, high-sensitivity MEA measurements are achieved following enzyme-mediated conversion of non-electroactive chemical species such as glutamate and glucose. (Table 1). These features make the MEA a highly sensitive and versatile neural device for spatially (*i.e.* micron-sized) and temporally (*i.e.* sub-second) resolved measures of numerous CNS neurotransmitters and neuromolecules. Because routine enzyme-based amperometric detection of glutamate using the same MEA has been achieved up to 17 days *in vivo* (Rutherford et al., 2007) and MEA-based single-unit neuronal recordings have been demonstrated at 8 weeks post-implantation (Moxon et al., 2004b), we were interested in evaluating the intrinsic physical properties of the MEA recording surface that may contribute to enhanced performance relative to other types of Pt electrodes (Table 1).

MEAs are constructed on inert ceramic (Al_2O_3), which has widespread use in orthopedic prostheses (Navarro et al., 2008) and dental implants (Kohal et al., 2008). The biocompatibility, durability, rigidity and strength of ceramic are important properties for machining purposes and make it highly desirable for use as an indwelling implant in living tissue. The insulating property of inert ceramic also reduces cross talk from adjacent connecting lines, making it of further utility for amperometric measurements (Burmeister et al., 2000). Although the integration of ceramic substrates in peripheral tissues is well documented, the biocompatibility of ceramic has only recently been considered in the CNS (Moxon et al., 2007; Hascup et al., 2009; Moxon et al., 2009). The minimally invasive ceramic-based MEAs caused minor tissue responsiveness in comparison to silicon-based

microelectrodes and microdialysis probes. Thus, it has become important to consider the post-fabrication topography of the ceramic substrate, as this will be key to understanding how the MEA integrates with neural tissue.

To date, the composition and characterization of thin film Pt recording sites, as well as the topography of the ceramic substrate, have not been demonstrated. Indeed, knowledge regarding these fundamental properties prior to implantation could be informative towards understanding the high-sensitivity recordings achieved with MEAs (Table 1). Here, we report on the intrinsic physical properties of the MEAs using complementary investigative techniques, which demonstrate nanoscale topography for the Pt recording sites and ceramic substrate. These studies are the first to describe the surface properties of an implantable, ceramic-based neural device and are a necessary step towards further minimizing tissue responsiveness, improving long-term implant success and enhancing signal-to-noise of implantable devices for CNS recordings.

2. Materials and Methods

2.1. MEA design and fabrication

S2 microelectrodes were used in the present study and consisted of an array of four Pt recording sites ($15\mu\text{m} \times 333\mu\text{m}$), which were geometrically arranged as two side-by-side pairs ($100\mu\text{m}$ between pairs, $30\mu\text{m}$ between sites within a pair). Connecting lines and bonding pads extended $\sim 1\text{ cm}$ from the recording sites. The design and fabrication of MEAs has been previously described in detail (Burmeister et al., 2000; Burmeister and Gerhardt, 2001; Hascup et al., 2007) and are briefly mentioned here. Photolithographic methods for the mass fabrication of MEAs were carried out in conjunction with Hybrid Circuits, Inc. (Sunnyvale, CA). Microelectrode images (including recording sites, connecting lines and bonding pads) were patterned onto a photoresist-coated $2.5\text{ cm} \times 2.5\text{ cm}$ ($125\mu\text{m}$ thick) ceramic substrate (Coors Ceramic Company, Golden, CO) by exposing the photoresist with collimated UV light through a custom-designed mask. Recording sites, connecting lines and bonding pads were defined by using a vacuum-sealed sputter chamber to sputter-coat the ceramic substrate with a $500\text{ \AA}$ adhesion layer of titanium and $2300\text{ \AA}$ layer of elemental Pt (Pt^0). With the exception of the Pt recording sites, all circuitry was insulated with a $1.2\mu\text{m}$ layer of polyimide to protect against aqueous environments and reduce cross-talk between connecting lines. Individual microelectrodes were sawed from patterned wafers using a computer-controlled diamond saw and were connected to printed circuit boards.

2.2. Scanning electron microscopy

Scanning electron microscopy was performed using a Hitachi S3200 variable pressure scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA). High-resolution electron micrographs of MEA ($n = 25$) surfaces were acquired using secondary electron imaging to capture surface features with high resolution. In some cases, backscattered electron detection was utilized to evaluate changes in MEA surface composition (*e.g.* recording site vs. polyimide layer). Conductive carbon adhesive tabs (0.5 in diameter; Electron Microscopy Science, Hatfield, PA) were used to ground the MEA surface while securing the sample to the specimen holder. An Emscope gold sputtering coater (Denton Vacuum, LLC, Moorestown, NJ) was used to metalize MEA surfaces with gold/palladium alloy. In turn, this process maximized the spatial resolution of the signal by preventing the accumulation of static electrical charge during exposure to the electron beam. The following operational parameters were used: accelerating voltage, 20 keV ; working distance, 15 mm ; emission current, $43\mu\text{A}$. Electron micrographs of the MEA surface were digitally captured, displayed and stored using Quartz PCI software (Hitachi High Technologies America, Pleasanton, CA). For scanning electron micrographs of the front

surface of the MEAs, the MEA was rotated in the X–Y plane and tilted with respect to the incident beam such that the images were maintained in focus while maximizing the yield of textural information. For energy dispersive spectroscopy studies, the Hitachi S3200 was equipped with a Noran XEDS (X-ray energy dispersive spectroscopy) system and was used with backscattered electron detection to conduct spot analysis of the Pt sites.

2.3. Atomic force microscopy

The topography of MEA Pt recording sites ($n = 9$) and back ceramic surfaces ($n = 6$) was measured by atomic force microscopy using a Veeco MultiMode nanoscope (Veeco Instruments, Inc., Plainview, NY). All MEA samples were studied at 25°C under normal atmospheric pressure. A standard, uncoated contact-mode silicon atomic force microscopy probe with a tip radius of ~10 nm and half cone angle (measured along the cantilever axis) ranging from 20 – 25° was used in the current study (Ted Pella, Inc., Redding, CA). The nanoscope maintained the probe in a fixed position while allowing the sample to move beneath the cantilever using a 2.4 Hz scan rate in a rastered fashion. The sampling area of the surface being measured was either 1 or 225 μm^2 . After obtaining three-dimensional surface profiles, three-dimensional images were constructed in perspective mode to reveal the shape and distribution of peaks and indentations across the sampled region or profile view mode (data not shown) to highlight feature-to-feature variability regarding lateral spread and vertical displacement. The height scale and deflection range for each image were adjusted to optimize the visual presentation of topographical information.

Atomic force microscopy data were quantified using roughness analysis for each MEA with scanning probe imaging processor software (SPIP v.5.1.3; Imagemet Metrology A/S, Denmark). The following parameters were calculated for each sampled surface with regards to the roughness analyses: average roughness (R_{AVG} , mean of absolute roughness values of peaks and indentations within the selected area); root mean squared roughness (R_{RMS}); surface symmetry (R_{SKEW} , dimensionless number signifying the relative proportion of peaks and indentations); maximum peak height within a sample (H_{MAX}); absolute maximum indentation depth within a sample ($|D_{\text{MAX}}|$). Values are reported as mean $\pm$ SEM for each of these parameters. Portions of an image with a sudden change in sample height (*e.g.* the boundary of a site or edge of the ceramic base) were expected to skew roughness values and, therefore, were excluded from the analyses. Line scan analyses of the Pt recording sites were performed as a qualitative assessment of surface roughness and to obtain a frequency distribution of the total number of surface features in terms of height or depth relative to the baseline surface.

3. Results and Discussion

3.1. Elemental composition of microelectrode array platinum recording sites

Knowledge regarding the elemental identity of the microelectrode recording sites can be informative for detecting potential contaminants and evaluating electrochemical behavior. To address this, we analyzed the elemental composition of the MEA recording sites with energy dispersive x-ray spectroscopy (Fig. 1). Spectral analysis confirmed that the recording sites are predominantly composed of Pt. The presence of multiple Pt peaks resulted from excitation energy levels arising from different electron orbitals. A peak corresponding to carbon was also observed. Although residual patterning material and/or impurities in the sputter chamber during the MEA fabrication process are potential sources of carbon, carbon may also be deposited from hydrocarbon contamination in the scanning electron microscopy chamber. Carbon has been shown to impede the amperometric detection of H_2O_2 (Ikariyama et al., 1988; Arbault et al., 1995). However, this is not the case here as functional tests have demonstrated that the MEA Pt recording sites are highly sensitive to H_2O_2 (Table 1).

Periodic assessment of MEA recording sites may be a useful exercise for determining whether such contaminants are within the tolerance range of the device.

The detection of H_2O_2 at Pt-based electrodes is believed to result from a catalytic reaction with a thin layer of Pt-oxide (Anson and Lingane, 1957; Lingane, 1961; Lingane and Lingane, 1963), and the resulting current corresponds to the re-oxidation of Pt to Pt-oxide following H_2O_2 -mediated reduction of the oxide film (Lingane and Lingane, 1963). Since the experimental approach used here does not provide direct information regarding oxygen presence or elemental oxidation state, future studies using advanced physical chemistry techniques that we presently do not have access to are necessary to determine if Pt-oxygen complexes are present on the recording surface before and after its polarization in an aqueous environment. If the MEA, thin film Pt recording sites have a unique physical chemistry for the catalytic reaction of H_2O_2 , such efforts may identify an important contributor towards explaining the enhanced sensitivity of the MEA for H_2O_2 -based measures of CNS neurotransmitters.

3.2. Photolithography produces recording sites with microwell geometries

The ceramic substrate, Pt recording sites and polyimide layer were distinguishable based on gray intensity levels using high-resolution secondary electron micrographs (Fig. 2A). The insulating layer of polyimide had a uniform surface composition and did not cover the Pt recording sites (Fig. 2A). Differences in surface composition on the front surface of the MEA were also confirmed through backscattered electron detection (data not shown). The Pt recording sites were further investigated using higher magnification secondary electron micrographs (Fig. 2B). Pt recording sites had a recessed appearance due to the polyimide around the recording pads, which created a microwell structure (Fig. 2B). Higher magnification micrographs revealed that the bare Pt recording sites contained irregularities in the form of elevations and depressions (Fig. 2C and 2D). The cross-sectional areas of these heterogeneous markings ranged from $0.04 - 25.06 \mu\text{m}^2$.

Application of exclusion layers and immobilized enzymes reduces electrode responsiveness to interferents (*e.g.* ascorbic acid) and enables the detection of non-electroactive neuromolecules, respectively (Burmeister et al., 2000; Burmeister and Gerhardt, 2001; Burmeister et al., 2008). The visualization of the microwell structure post-fabrication is an exciting finding in these studies as it is key to improving MEA coatings (*i.e.* exclusion layers and immobilized enzyme(s)). Protein attachment or chemical adsorption to microwell structures has primarily been studied in the context of cell culturing and high-throughput immunoassays. Comparisons between micron-sized plates and wells have demonstrated superior protein immobilization using the latter design (Jung et al., 2008). Enzyme activity and composition mixtures are important considerations for enzyme-based sensors and have been optimized through parametric studies for MEA-based measures of glutamate (Hascup et al., 2007). In turn, the amount and targeting of the applied enzyme solution to the MEA surface has been a continued area of investigation by our laboratory. Enzyme solutions are currently applied overselected recording sites with the aid of a dissection microscope (Hascup et al., 2007). While the hand-applied enzyme solutions are targeted with great precision to selected Pt recording site(s) in the microwell region, the immobilized enzyme is not exclusive to the Pt recording site(s) - owing to an applied droplet size that is much larger than a single recording site. Thus, enzyme solution also covers adjacent, polyimide-layered ceramic substrate. Because newly generated H_2O_2 does not diffuse appreciably to enzyme-free (*i.e.* sentinel) recording sites following the local application of glutamate near the MEA *in vivo* (Rutherford et al., 2007; Hascup et al., 2008), we hypothesize that the enzyme molecules in closest proximity to the recording site provide H_2O_2 reporter molecules for subsequent detection. The advantage of a precisely defined microwell should benefit our goal of applying enzyme coatings with greater precision. The use of microprinting

technology is under development and is aimed at providing a more controlled delivery of enzyme - restricting it to the microwell structure. These developments will be instrumental to further assessing the contribution of the applied enzyme amount (*i.e.* enzyme coverage area) to MEA sensitivity and to improving coating stability for extended periods post-implantation.

Stable adhesion of coatings to the MEA recording surfaces is important for monitoring neuromolecules in both acute and chronic settings. In recent years, our attention has shifted towards freely-moving recordings as anesthetic-mediated effects can be avoided and longitudinal changes can be monitored using animal models of disease (Rutherford et al., 2007). Decreased signal-to-noise has restricted enzyme-based amperometric glutamate detection using the same MEA to 17 days post-implantation (Rutherford et al., 2007). However, MEA-based single-unit neuronal recordings have been demonstrated at 8 weeks post-implantation (Moxon et al., 2004b), suggesting that impaired glutamate detection likely arises from changes in the extracellular environment, exclusion layers and/or immobilized enzyme rather than a fault in the basic recording properties of the Pt sites. Thus, future studies are needed to determine how the brain microenvironment and tissue responsiveness directly affect immobilized enzyme layers. Parameters such as the depth of the microwell, distance between electrodes on a multi-site array and software limitations must also be considered for improving both acute and chronic recording performance (Sandison et al., 2002).

3.3. Microelectrode array platinum recording sites have nanoscale topography

Because secondary electron micrographs revealed heterogeneous, irregular markings on the bare Pt recording surface and previous studies have shown that nanostructured surfaces can improve electrode performance (Han et al., 2006), we determined the topography of the recording surface at the microscale and nanoscale levels. Atomic force microscopy was employed to further examine the topography of Pt recording sites, including information regarding surface height and roughness. By using the same atomic force microscopy probe, we were able to maintain identical spatial resolution while sampling different surface areas of the Pt recording sites. In addition, different sampling areas allowed surface details to be visualized at different magnifications while isolating regions of interest. The topographies of Pt recording sites from 225 μm^2 and 1 μm^2 sampled areas are shown in Fig. 2E and 2F, respectively. Lighter shading is indicative of regions with raised topography while darker shading corresponds to indented areas.

The surface characteristics of the Pt recording sites were further extended through roughness analyses (Table 2). R_{AVG} and R_{RMS} values described a recording surface that is rough at the nanoscale level for both 225 μm^2 and 1 μm^2 sampling areas. The R_{SKEW} value, a measure of the relative proportion of elevations and depressions, obtained for 225 μm^2 sampled areas described an asymmetric surface containing more peaks than indentations (*i.e.* $R_{\text{SKEW}} \geq 1$). R_{SKEW} values for 1 μm^2 sampled areas were suggestive of a surface mostly containing small indentations. These findings were further supported by the values obtained for H_{MAX} (maximum peak height) and $|D_{\text{MAX}}|$ (absolute maximum depth of indentations). Collectively, these data support corresponding secondary electron micrographs and verify the non-uniform, nanoscale topography of the Pt recording surfaces.

Roughness analysis has several noteworthy limitations. First, the calculations for R_{AVG} and R_{RMS} do not take into consideration sample surface indentations that are not fully resolved. Second, these values can be distorted by outlier peaks of extreme height (*e.g.* features in the height range of H_{MAX}) and do not distinguish between the distribution and frequency of peaks. Finally, R_{SKEW} does not account for the actual distribution of peaks and indentations. Therefore, the roughness values for two sites can be identical even if the peaks are grouped

together on one site but are distributed evenly on the other. To circumvent many of these issues we examined the surface height across multiple cross-sections of each site through line scan analyses (Fig. 3). Overall, the profiles for each site indicated that the topography of the recording surfaces is dominated by nanometer sized elevations and depressions (absolute height < 5 nm; Fig. 3A). Extreme peaks and holes occurred less frequently, and the lateral distribution of extreme surface features was unique for each site. The total number and height of surface features on each site was obtained by constructing a frequency distribution (Fig. 3B). While absolute values were variable between sampled lines (data not shown), the frequency distribution demonstrated near-Gaussian properties for every sample. In addition, the majority of data points were within an absolute height value of 10 nm from the mean feature height. Note that the distribution also indicates flat regions (height = 0).

To our knowledge, the influence of processing conditions on recording lifetime and H₂O₂ sensitivity has not been performed for different Pt electrodes. Table 1 shows substantial differences in electrode responsiveness that cannot be explained by changes in electrode geometry alone as sensitivity per unit area for H₂O₂, glutamate and glucose varies substantially between different types of Pt-based electrodes. While Table 1 compares the MEA device to other commonly used Pt-based electrodes for given analytes, it is important to recognize that other high-sensitivity Pt, enzyme-based sensors have been reported. For example, high-sensitivity (2500 pA·μM⁻¹·mm⁻²) ATP biosensors that generate H₂O₂ reporter molecules have had been successfully used *in vivo* (Laudet et al., 2005). Thus, it will be interesting to see how MEAs compare with other established biosensors as we begin to expand the recording capabilities of the device to enzyme-based measures of other analytes.

Evaluation of MEA performance supports a highly active recording surface that rivals or exceeds the sensitivity previously reported by many groups (Table 1). The disconnect between analyte sensitivity between electrode subtypes may be the result of differences in material selection and/or fabrication processes. To the best of our knowledge, neither has been directly investigated through systematic studies as it requires a cooperative, group effort among different electrode users. The nanoscale topography of the MEA recording sites produces an electroactive area that is greater than the geometric area. These findings support that sensitivity can be higher than that for an ideal planar electrode, if the current is not limited by diffusion and all the active recording sites can be accessed. If a molecule cannot reach a narrow cavity or a steep wall, these regions represent areas of less than average reaction probability. Thus, total surface area does not necessarily translate to total accessible area. Subtle differences in recording surface nanofeatures may also contribute to differences in recording performance between individual recording sites.

3.4. Microelectrode array ceramic topography

While the Pt recording sites on the front face of the MEA are necessary for electrochemical detection, the ceramic substrate is an important consideration regarding chronic implantation *in vivo*. To better understand the topography of the ceramic substrate, secondary electron micrographs of the back (Fig. 4A and 4B) and side (Fig. 4A, 4C and 4D) surfaces of the MEA were captured. The topographical hallmarks on the back surface of the MEAs (Fig. 4B) included intersecting microgrooves (width < 1 μm) and focal pits with cross-sectional areas that ranged between 0.1 – 100 μm². Both features likely arise from the polishing of the ceramic substrate, the discussion of which has been reviewed elsewhere (Zarudi et al., 1998; Ravikiran, 1999, 2000a, b). The ceramic-based sides and tip of the MEAs were composed of an extensive network of interconnected ridges and cavities, which contributed to a unique, rough appearance (Fig. 2A and 4C). Boundaries between individual ridges and cavities were not readily distinguishable, making detailed cross-sectional measurements of individual surface features not possible. In general, larger surface cavities were composed of clusters of

smaller microfeatures and connected to nearby cavities through fissures and surface corrugations (Fig. 4D). The increased brightness observed along the ridges of some cavities reflects a sharp change in sample height/depth and enhanced secondary electron emission.

Because the ceramic on the sides of the MEA had a high level of roughness and could potentially cause probe damage to the atomic force microscopy tips, atomic force microscopy was used only to map ceramic surfaces on the back of the MEAs, which were polished (Table 2). The inner surfaces of the microgrooves observed on the back of the MEA were irregular in shape but were relatively consistent in maximum depth ($|D_{MAX}|$) for the sampled areas in this study. Extreme values for $|D_{MAX}|$ and H_{MAX} here were 201 nm and 688 nm, respectively. R_{SKEW} and height distribution data (data not shown) indicated that the microgrooves were the dominating surface feature. R_{AVG} and R_{RMS} values were extreme and not meaningful on the front surface of the MEA due to height variation between the ceramic substrate, polyimide layer, connecting lines and Pt recording sites and, therefore, were not further investigated.

The topography of an indwelling neural device can greatly influence its biocompatibility and post-implantation success rate (Polikov et al., 2005). Thus, strategies that increase the porosity of the microelectrode substrate are conducive to biocompatibility and long-term implant success (Moxon et al., 2004a; Moxon et al., 2007; Moxon et al., 2009). Indeed, by altering the roughness of the ceramic surface, improved rates of implant success have been achieved (Wennerberg and Albrektsson, 2010). Because adverse responses from surrounding brain tissue are minimal with our MEA device weeks to months post-implantation (Hascup et al., 2009), it is likely that the small size of the MEA taken together with the unique qualities of the ceramic surface make it suitable for integration in host tissues. Future studies are necessary to determine whether biological tissues adhere to the MEA surface, if the different surface topographies translate to differential tissue adherence on the front, back and side ceramic surfaces and the extent to which the brain microenvironment affects immobilized enzymes. These studies will be helpful towards improving the sensitivity and stability of the MEA recordings.

4. Conclusions

In addition to extensive use in rodents, MEAs have been used for the intracerebral monitoring of neurochemicals in translational models such as non-human primates (Quintero et al., 2007; Stephens et al., 2008; Stephens et al., 2010). As such, we continue to evaluate aspects of the technology that can be modified to provide more stable and sensitive recordings of neurochemicals. When viewed under the optical microscope, machined surfaces and Pt recording sites of MEAs appear smooth and light reflective. These studies are the first to describe the surface properties of an implantable ceramic-based neural device, which has important implications towards the future development/improvement of the MEA technology while serving as a comparison for other types of intracerebral neural devices. Here, we demonstrated that individual aspects of the MEA device retain unique physical properties post-fabrication. The characteristic surface chemistry, microwell geometry and nanoscale features of MEAs are favorable for high sensitivity recordings. In addition, the nanoscale roughness of the inert ceramic substrate is, in part, likely a contributing factor to post-implantation success. While *in vivo* enzyme-based measures of glutamate have been restricted to 17 days post-implantation (Rutherford et al., 2007), MEA-based single-unit recordings have been obtained up to 8 weeks *in vivo* (Moxon et al., 2004b). Thus, the presence of nanoscale features on the Pt recording sites, which contribute to an enhanced electroactive area, have emerged as a primary contributor to the high sensitivity performance of the MEA. In addition, approaches aimed at enhancing the nanoscale features of the Pt recording sites may prove useful towards enhancing the recording performance of the device

(Han et al., 2006). The continued refinement of enzyme immobilization approaches, including restriction of enzyme solution to the microwell and identifying factors that affect enzyme performance following prolonged implantation *in vivo*, will be essential to further optimizing enzyme-coating conditions. The present study is instrumental to ongoing efforts aimed at improving and extending the recording performance of the ceramic-based MEAs.

Acknowledgments

We thank Larry Rice and George Turpin (University of Kentucky Electron Microscopy Center, Lexington, KY) for their assistance with data processing. This work was supported by grants from USPHS NS39787, DA017186, AG13494, T32 AG000242 and NSF EEC-0310723.

References

- Anson FC, Lingane JJ. Chemical evidence for oxide films on platinum electrometric electrodes. *J Am Chem Soc.* 1957; 79:4901–4.
- Arbault S, Pantano P, Jankowski JA, Vuillaume M, Amatore C. Monitoring an oxidative stress mechanism at a single human fibroblast. *Anal Chem.* 1995; 67:3382–90. [PubMed: 8686890]
- Burmeister JJ, Gerhardt GA. Self-referencing ceramic-based multisite microelectrodes for the detection and elimination of interferences from the measurement of L-glutamate and other analytes. *Anal Chem.* 2001; 73:1037–42. [PubMed: 11289414]
- Burmeister, JJ.; Gerhardt, GA. Neurochemical arrays. In: Grimes, C.; Dickey, E.; Pishko, MV., editors. *Encyclopedia of Sensors*. American Scientific Publishers; Stevenson Ranch: 2006. p. 525–37.
- Burmeister JJ, Moxon K, Gerhardt GA. Ceramic-based multisite microelectrodes for electrochemical recordings. *Anal Chem.* 2000; 72:187–92. [PubMed: 10655652]
- Burmeister JJ, Pomerleau F, Huettl P, Gash CR, Werner CE, Bruno JP, Gerhardt GA. Ceramic-based multisite microelectrode arrays for simultaneous measures of choline and acetylcholine in CNS. *Biosens Bioelectron.* 2008; 23:1382–9. [PubMed: 18243683]
- Gerhardt, GA.; Burmeister, JJ. *In vivo* voltammetry for chemical analysis of the nervous system. In: Meyers, RA., editor. *Encyclopedia of Analytical Chemistry: Instrumentation and Applications*. John Wiley and Sons; Chichester: 2000. p. 710–31.
- Hamdi N, Wang J, Walker E, Maidment NT, Monbouquette HG. An electroenzymatic l-glutamate microbiosensor selective against dopamine. *J Electroanal Chem.* 2006; 591:33–40.
- Han J-H, Boo H, Park S, Chung TD. Electrochemical oxidation of hydrogen peroxide at nanoporous platinum electrodes and the application to glutamate microsensor. *Electrochimica Acta.* 2006; 52:1788–91.
- Hascup ER, af Bjerkén S, Hascup KN, Pomerleau F, Huettl P, Strömberg I, Gerhardt GA. Histological studies of the effects of chronic implantation of ceramic-based microelectrode arrays and microdialysis probes in rat prefrontal cortex. *Brain Res.* 2009; 1291:12–20. [PubMed: 19577548]
- Hascup KN, Hascup ER, Pomerleau F, Huettl P, Gerhardt GA. Second-by-second measures of L-glutamate in the prefrontal cortex and striatum of freely moving mice. *J Pharmacol Exp Therap.* 2008; 324:725–731. [PubMed: 18024788]
- Hascup, KN.; Rutherford, EC.; Quintero, JE.; Day, BK.; Nickell, JR.; Pomerleau, F.; Huettl, P.; Burmeister, JJ.; Gerhardt, GA. Second-by-second measures of L-glutamate and other neurotransmitters using enzyme-based microelectrode arrays. In: Michael, AC.; Borland, LM., editors. *Electrochemical Methods for Neuroscience*. CRC Press; Boca Raton: 2007. p. 407–50.
- Ikariyama Y, Yamauchi S, Yukiashi T, Ushioda H. Surface control of platinized platinum as a transducer matrix for micro-enzyme electrodes. *J Electroanal Chem Interfacial Electrochem.* 1988; 251:267–74.
- Jung E, Manassis D, Neumann A, Böttcher L, Braun T, Bauer J, Reichl H, Iafelice B, Destro F, Gambari R. Lamination and laser structuring for a microwell array. *Microsystem Technologies.* 2008; 14:931–6.

- Kim CS, Oh SM. Enzyme sensors prepared by electro deposition on platinized platinum electrodes. *Electrochimica Acta*. 1996; 41:2433–9.
- Kohal R-J, Att W, Bächle M, Butz F. Ceramic abutments and ceramic oral implants: an update. *Periodontology*. 2008; 47:224–43.
- Lingane JJ. Chronopotentiometric study of oxygen reduction at a platinum wire cathode. *J Electroanal Chem*. 1961; 2:296–309.
- Lingane JJ, Lingane PJ. Chronopotentiometry of hydrogen peroxide with a platinum wire electrode. *J Electroanal Chem*. 1963:411–9.
- Llaudet E, Hatz S, Droniou M, Dale N. Microelectrode biosensor for real-time measurement of ATP in biological tissue. *Anal Chem*. 2005; 77:3267–73. [PubMed: 15889918]
- Moxon KA, Hallman S, Aslani A, Kalkhoran NM, Lelkes PI. Bioactive properties of nanostructured porous silicon for enhancing electrode to neuron interfaces. *J Biomater Sci Polym Ed*. 2007; 18:1263–81. [PubMed: 17939885]
- Moxon KA, Hallman S, Sundarakrishnan A, Wheatley M, Nissanov J, Barbee KA. Long-term recording of multiple, single-neurons for clinical applications: the emerging role of the bioactive microelectrode. *Materials*. 2009; 2:1762–94.
- Moxon KA, Kalkhoran NM, Markert M, Sambito MA, McKenzie JL, Webster JT. Nanostructured surface modification of ceramic-based microelectrodes to enhance biocompatibility for a direct brain-machine interface. *IEEE Trans Biomed Eng*. 2004a; 51:881–9. [PubMed: 15188854]
- Moxon KA, Leiser SC, Gerhardt GA, Barbee KA, Chapin JK. Ceramic-based multisite electrode arrays for chronic single-neuron recording. *IEEE Trans Biomed Eng*. 2004b; 51:647–56. [PubMed: 15072219]
- Navarro M, Michiardi A, Castaño O, Planell JA. Biomaterials in orthopaedics. *J R Soc Interface*. 2008;5.
- O'Neill RD, Chang S-C, Lowry JP, McNeil CJ. Comparisons of platinum, gold, palladium and glassy carbon as electrode materials in the design of biosensors for glutamate. *Biosens Bioelectron*. 2004; 19:1521–8. [PubMed: 15093225]
- Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. *J Neurosci Methods*. 2005; 148:1–18. [PubMed: 16198003]
- Quintero JE, Day BK, Zhang Z, Grondin R, Stephens ML, Huettl P, Pomerleau F, Gash DM, Gerhardt GA. Amperometric measures of age-related changes in glutamate regulation in the cortex of rhesus monkeys. *Exp Neurol*. 2007:238–46. [PubMed: 17927982]
- Ravikiran A. Transformation of polishing grooves into ridges on the worn surface of alumina. *J Am Ceram Soc*. 1999; 82:261.
- Ravikiran A. Effect of sliding conditions on formation of grain-pits due to wear anisotropy. *J Mater Sci Lett*. 2000a; 19:1041–3.
- Ravikiran A. Wear mechanism based on wear anisotropy. *Tribol Trans*. 2000b; 43:287–92.
- Reyes De Corcuera JI, Cavalieri RP, Powers JR. Improved platinization conditions produce a 60-fold increase in sensitivity of amperometric biosensors using glucose oxidase immobilized in poly-o-phenylenediamine. *J Electroanal Chem*. 2005; 575:229–41.
- Rutherford EC, Pomerleau F, Huettl P, Strömberg I, Gerhardt GA. Chronic second-by-second measures of l-glutamate in the central nervous system of freely moving rats. *J Neurochem*. 2007; 102:712–22. [PubMed: 17630982]
- Sandison ME, Anicet N, Glidle A, Cooper JM. Optimization of the geometry and porosity of microelectrode arrays for sensor design. *Anal Chem*. 2002; 74:5717–25. [PubMed: 12463354]
- Stephens ML, Pomerleau F, Huettl P, Gerhardt GA, Zhang Z. Real-time glutamate measurements in the putamen of awake rhesus monkeys using an enzyme-based human microelectrode array prototype. *J Neurosci Methods*. 2010; 185:264–72. [PubMed: 19850078]
- Stephens ML, Quintero JE, Pomerleau F, Huettl P, Gerhardt GA. Age-related changes in glutamate release in the CA3 and dentate gyrus of the rat hippocampus. *Neurobiol Aging*. In Press.
- Stephens, ML.; Spencer, DD.; Cavus, I.; Hsiao, M.; Song, D.; Deadwyler, SA.; Hampson, RE.; Putz, D.; Quintero, JE.; Bensalem-Owen, MK.; Hascup, KN.; Rutherford, EC.; Day, BK.; Nickell, JR.; Pomerleau, F.; Huettl, P.; Burmeister, JJ.; Talauliker, PM.; Marmarelis, VZ.; Granacki, JJ.; Berger, T.; Gerhardt, GA. Microelectrode-based epilepsy therapy: a hybrid neural prosthesis

incorporating seizure prediction and intervention with biomimetic maintenance of normal hippocampal function. In: Soltesz, I.; Staley, K., editors. *Computational Neuroscience in Epilepsy*. Elsevier Academic Press; Amsterdam: 2008. p. 540-63.

Wang J, Myung NV, Yun M, Monbouquette HG. Glucose oxidase entrapped in polypyrrole on high-surface-area Pt electrodes: a model platform for sensitive electroenzymatic biosensors. *J Electroanal Chem*. 2005; 575:139–46.

Wennerberg A, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. *International Journal of Oral Maxillofacial Implants*. 2010; 25:63–74. [PubMed: 20209188]

You T, Niwa O, Tomita M, Hirano S. Characterization of platinum nanoparticle-embedded carbon film electrode and its detection of hydrogen peroxide. *Anal Chem*. 2003; 75:2080–5. [PubMed: 12720344]

Zarudi I, Zhang L, Cockayne D. Subsurface structure of alumina associated with single-point scratching. *J Mater Sci*. 1998; 33:1639–45.

Research Highlights

- The physical properties of an implantable, ceramic neural device are described.
- Microelectrode array recording sites are primarily composed of elemental platinum.
- Platinum recording sites sit in a microwell and have nanoscale surface topography.
- The inert ceramic substrate has nanoscale roughness.
- The device's unique physical chemistry likely contributes to *in vivo* performance.

Fig. 1. Microelectrode array recording sites are primarily composed of elemental platinum. Energy dispersive spectroscopy was utilized to reveal the elemental composition of MEA recording sites. A representative spectrum is shown. Multiple peaks for Pt correspond to excitation energies of different orbitals. C, carbon; Pt, platinum.

Fig. 2. Surface characterization of microelectrode array platinum recording sites. (A) Representative secondary electron micrograph of the front and side surfaces of a MEA (rotation angle = 315° ; tilt angle = 16°). Representative high magnification secondary electron micrographs of (B) the front surface of a MEA (rotation angle = 50° ; tilt angle = -10°) and (C) a Pt recording site (rotation angle = 0° ; tilt angle = 0°). (D) Representative higher magnification secondary electron micrograph demonstrating sub-micron sized elevations (solid boxes) and depressions (dashed boxes) on the bare Pt recording site (rotation angle = 0° ; tilt angle = 0°). Three-dimensional perspective projections of Pt recording sites obtained with atomic force microscopy of (E) $225 \mu\text{m}^2$ (deflection range = 42.59 nm) and (F) $1 \mu\text{m}^2$ (deflection range = 1.39 nm) sampled areas.

Fig. 3. Line scan analysis of microelectrode array platinum recording sites. (A) Line locations for multiple line scans on a representative $225 \mu\text{m}^2$ sampled area with corresponding line profiles are shown. (B) Frequency distribution graph showing the total number of surface features for the sampled Pt recording site shown in (A) in terms of surface height and depth.

Fig. 4. Surface characterization of the microelectrode array ceramic substrate. (A) Representative secondary electron micrograph of the ceramic surface on the back, side and tip of the MEA (rotation angle = 41°; tilt angle = -10°). Representative high magnification secondary electron micrographs of the ceramic substrate on (B) the back (rotation angle = 0°; tilt angle = 0°) and (C) side and tip (rotation angle = 35°; tilt angle = 35°) of the MEA. (D) Representative higher magnification secondary electron micrograph of microfeatures on the ceramic surface on the side of the MEA shown in (C) (rotation angle = 35°; tilt angle = 35°).

Table 1

Sensitivity per unit area comparisons for platinum-based measures of different analytes.

Analyte measured	Electrode type	Geometric area (mm ²)	Sensitivity (pA·μM ⁻¹ ·mm ⁻²)	Reference
H ₂ O ₂	Thin film MEA	0.0025	4880	Burmeister et al., 2000
H ₂ O ₂	Disk	2.0	554	O'Neill et al., 2004
H ₂ O ₂	Disk Pt-NEGCF	7.1	550 ^b	You et al., 2003
H ₂ O ₂	Disk ^a	490	490	Han et al., 2006
Glutamate	Thin film MEA	0.005	1201	Stephens et al., in press
Glutamate	Disk	2.0	268	O'Neill et al., 2004
Glutamate	Wire ^a	0.1 [†]	800	Hamdi et al., 2006
Glucose	Thin film MEA	0.005	2338	* Current study
Glucose	Wire ^a	11.0	1920 ^c	Reyes De Corcuera et al., 2005
Glucose	Disk ^a	1.2	16.7 ^b	Kim and Oh, 1996
Glucose	Thin film ^a	1.0	1028 ^c	Wang et al., 2005

Pt-NEGCF, Pt nanoparticles embedded in graphite-like carbon film.

^aPlatinized electrode,^bestimated from data,^cmaximum reported value

* Current study was carried out exactly as described (Burmeister et al., 2001) using S2 MEAs without polyurethane.

Table 2

Roughness analysis of platinum recording sites and the back ceramic surface.

	R_{AVG} (nm)	R_{RMS} (nm)	R_{SKEW}	H_{MAX} (nm)	 D_{MAX} (nm)
Platinum					
225 μm ²	8.59 ± 0.25	11.77 ± 0.21	1.00 ± 0.51	170.74 ± 9.97	192.32 ± 5.51
1 μm ²	4.73 ± 0.31	6.56 ± 0.53	-0.37 ± 0.64	22.87 ± 8.20	21.89 ± 0.43
Ceramic					
225 μm ²	19.35 ± 8.44	23.8 ± 4.10	-1.24 ± 0.6	392.87 ± 132.14	176.11 ± 11.02

Data were quantified from n = 9 platinum recording sites for 225 or 1 μm² sampled areas and n = 6 ceramic surfaces (back of the MEA) for 225 μm² sampled areas.