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Chronic Intracortical Microelectrode Arrays Induce Non-Uniform, Depth-Related Tissue Responses

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Abstract

Objective—Brain-implanted microelectrode arrays show promise as future clinical devices. However, biological responses to various designs, compositions, and locations of these implants have not been fully characterized, and may impact the long term functionality of these devices. In order to improve our understanding of the tissue conditions at the interface of chronic brain-implanted microdevices, we proposed utilizing advanced histology and microscopy techniques to image implanted devices and surrounding tissue intact within brain slices. We then proposed utilizing these methods to examine whether depth within the cerebral cortex affected tissue conditions around implants.

Approach—Histological data was collected from rodent brain slices containing intact, intracortical microdevices 4 weeks after implantation surgery. Thick tissue sections containing the chronic implants were processed with fluorescent antibody labels, and imaged in an optical clearing solution using laser confocal microscopy.

Main Results—Tissue surrounding microdevices exhibited two major depth-related phenomena: a non-uniform microglial coating along the device length and a dense mass of cells surrounding the implant in cerebral cortical layers I and II. Detailed views of the monocyte-derived immune cells improve our understanding of the close and complex association that immune cells have with chronic brain-implants, and illuminated a possible relationship between cortical depth and the intensity of a chronic monocyte response penetrating microdevices. The dense mass of cells contained Vimentin, a protein typically expressed highly in non-CNS cells, evidence that non-CNS cells likely descended down the face of the penetrating devices from the pial surface.

Significance—Image data of highly non-uniform and depth-dependent biological responses along a device provides novel insight into the complexity of the tissue response to penetrating brain-implanted microdevices. The presented work also demonstrates the value of *in situ* histological collection of brain-implants for studying the complex tissue changes that occur, and the utility of pairing thick-tissue histology with appropriate optical clearing solutions.

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Keywords

glial encapsulation; microglia; brain machine interface; neuroprosthetics; in situ device imaging; histology; optical clearing solution; neural engineering

1. Introduction

Implantable microdevices hold the promise of providing selective, long-term interfaces between brain tissue and electronics (Drake *et al.*, 1988). Microelectrode arrays (MEAs) have been implanted into monkey and human subjects towards the development of treatments for such disparate disorders as paralysis and blindness (Troyk *et al.*, 2005; Hochberg *et al.*, 2006). However the long term performance of these devices in brain tissue appears to be affected by a complex foreign body response to the implants (Szarowski *et al.*, 2003; Polikov *et al.*, 2005; McConnell *et al.*, 2009), leading to decreasing device performance over time (Williams *et al.*, 1999; Vetter *et al.*, 2004). The origin of this tissue response and the degree to which it is similar to non-CNS foreign body responses is not well understood (see recent review by Leach *et al.*, 2010).

A major challenge to studying the brain tissue response around implanted microdevices is related to difficulties inherent in the histological processing of the tissue of interest. Brain-implanted microdevices are typically explanted prior to tissue sectioning; otherwise shattering and dragging of the devices can occur during tissue sectioning. However, removal of the device leads to morphological disturbance of the tissue directly interfacing with the surface of the device (Holecko *et al.*, 2005), with authors reporting tissue adherent to the surface of some explanted devices (Biran *et al.*, 2005). Distorted and incomplete histological data can thus complicate characterization of the biological conditions around intracortical implants, resulting in loose correlations between histological and physiological results.

In this article we present detailed histological data taken along the interfaces of intact MEAs, collected using a recently formalized method called Device Capture Histology, or DCHist (Woolley *et al.*, 2011). Commercially available single-shank devices, implanted into adult rat cerebral cortex for four weeks, were captured within thick sections of surrounding tissue (350 to 450 μm). After tissue collection, slices were labeled with fluorescent markers and imaged in a sucrose-based optical clearing solution (Tsai *et al.*, 2009). High resolution data sets were collected by laser scanning confocal microscopy.

The histological data collected in this study revealed complex, depth-related responses by microglia/macrophages along implanted devices. We also describe dense, tightly populated masses of cells, likely of non-CNS origin, surrounding superficial portions of the silicon device 4-weeks implanted. These masses did not contain substantial Iba1 signal, a monocyte marker, but did have a significant Vimentin expression, indicating a possible non-CNS, fibroblast-cell origin. With further application of the intact, *in situ* techniques utilized here we hope to better understand the origin of this cellular mass, and characterize its progression along long-term penetrating MEA interfaces. We also hope that further application of the methods presented here will serve to inform the development of improved brain-computer interface technologies.

2. Methods

To investigate the intact biological response around chronically brain-implanted MEAs (NeuroNexus Technologies), histological data was collected from rats implanted for 28 days with a single-shank MEA. The laboratory animal protocol for this work was approved through the Institutional Animal Care and Use Committee of Purdue University (West Lafayette, IN, USA), and conforms to the guidelines of the US National Institutes of Health.

2.1. Surgery

Aseptic surgeries were performed with sterilized tools and the aid of an assistant (Fig. 1c). Male Sprague-Dawley rats (6 total, 300 to 450 g, single-housed, Harlan Laboratories, Indianapolis, IN) were anesthetized with a cocktail of Ketamine (75-95 mg/kg) and Xylazine (5 mg/kg). Their heads were then shaved and swabbed with three alternating washes of Betadine and ethanol, ending with ethanol. Subjects were then placed on a warm-water circulation mat (T/Pump, Gaymar, Orchard Park, NY) resting on a steel plate on the surgical table. Eye lubricating ointment (Akwa Tears, Akorn Pharmaceutical, Lake Forest, IL) was applied. A pulse-oximeter (8600V, Nonin Medical, Plymouth, MN) was attached on a foot, and an oxygen mask was placed over the face. Sterile drape was laid over the subject, and a hole was cut to expose the shaved head. Lidocaine was injected subcutaneously at multiple positions on the head. Surgical assistants assessed the subject's level of anesthesia by toe pressure reflex at 30 minute intervals, delivering a bolus of 0.1 ml to 0.2 ml of Ketamine by intramuscular injection as necessary.

An approximately 2 cm midline incision was made along the midline of the cranium using a scalpel. The underlying muscle and connective tissue was retracted to expose the skull. A stainless steel bar was attached to the skull, anterior to bregma, using Loctite 454 (Henkle Corp, USA) and dental cement (Lang Dental Manufacturing Inc., Wheeling, IL). This sterilized bar was secured to a magnetic base (World Precision Instruments), which in turn was secured to a steel plate resting on the surgical table. A single craniotomy, roughly 2.5 mm in diameter, was made centered at approximately -1.5 mm posterior to bregma and 2.1 mm lateral to midline using a dental drill (Z-35, Henry Schein Inc., Melville, NY). Sterile saline was applied during pauses while drilling to help dissipate any local heating. The exposed dura was wetted with Gelfoam (Pfizer Inc., USA) and saline, and carefully incised at an area avoiding large surface vasculature using surgical Micro-scissors (Fine Science Tools, Foster City, CA).

In this experiment a single shank, unmounted microelectrode array (Fig. 1a) with 16 iridium electrodes arranged on a micromanufactured silicon substrate (Fig. 1b) was implanted into each animal. These devices were driven into exposed brain while held by vacuum-tipped tweezers (Pro-craft Pick-Up Set, Grobet USA, Carlstadt, NJ), mounted to a magnet-stabilized micromanipulator. After rapid insertion just beyond the final electrode site, the exposed shank and bonding pad was cut with microscissors and removed. Approximately 0.1 ml of warm, autoclave sterilized type III agarose (1% w/v in saline, Sigma-Aldrich Co., St. Louis, MO) was placed over the craniotomy through a 3 μ m syringe filter. The steel bar was removed and the incision was closed with nylon sutures (Ethicon Inc., Somerville, NJ). Triple antibiotic ointment was applied and 0.04 ml of Baytril (Bayer Corp., USA) in 3 ml of

sterile saline was delivered in two 1.5 ml subcutaneous injections. Once ambulatory, subjects were returned to their home cage and *ad libitum* food and water.

2.2. Perfusion, brain collection and tissue processing

Subjects under deep Ketamine/Xylazine anesthesia (95 mg/kg, 5 mg/kg, respectively) were perfused through the heart with Phosphate Buffered Saline (PBS) followed by a 4% formaldehyde solution (both pH 7.4), after which the head was removed and allowed to post-fix in formaldehyde for 24 hours at 4°C. To label vasculature, some subjects were given an intracardial perfusion of DiI (1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate) solution after PBS and before formaldehyde perfusion, as described in Li *et al.*, 2008. The removed heads were then washed 3 times over a 12 hour period in PBS, after which the brains were removed with care taken to avoid disturbing the implanted MEA. Brains were placed into HEPES Buffered Hank's Solutions with Sodium Azide (HBHS, pH 7.4; in g/L: 7.5 g NaCl, 0.3 g KCl, 0.06 g KH₂PO₄, 0.13 g Na₂HPO₄, 2 g Glucose, 2.4 g HEPES, 0.05 g MgCl₂·6H₂O, 0.05 g MgSO₄·7H₂O, 0.165 g CaCl₂, 90 mg NaN₃) and returned to 4°C until sectioning. Upon sectioning, a slicing plane was first created by placing the brain into a petri dish with PBS, and cutting the brain at a distance from the MEA in approximately the same plane as MEA implantation using a razor blade attached to a micromanipulator. This plane was then superglued to a vibratome stage and sectioned so as to collect the MEA within a slice of 350 to 450 µm of tissue (Fig. 1d). Control slices of similar thickness were also collected. This study focused primarily on labeling cell nuclei with Hoechst 33342 (Hoe342, 1:5000, Invitrogen, Green Island, NY), and labeling microglia/macrophage cells with a purified antibody to the protein Ionized calcium binding adaptor molecule 1 (Iba1, 1:100, Wako Chemical USA, Richmond, VA). In some subjects, labeling for Vimentin was performed (Vim, 1:100, Abcam, Cambridge, MA), to identify tissue of mesenchymal origin, or glial fibrillary acidic protein (GFAP, 1:100, Millipore Corp., Bellerica, MA) to identify astrocytes. After 5 to 7 days of incubation, tissue was washed repeatedly in wash solution (WS, 2% v/v normal goat serum, 0.2% v/v Triton-X 100 (Sigma-Aldrich), in HBHS) over 2 days, retuning to 4°C between washes. Alexa fluor conjugated secondary antibodies were used with Iba1 (Alexa fluors goat anti-rabbit 633 and goat anti-rabbit 488, each at 1:100, Invitrogen) and GFAP (Alexa Fluor goat anti-chicken 555, 1:100, Invitrogen) while DyLight secondary was used for Vim (DyLight 649 goat anti-chicken, 1:100, Jackson ImmunoResearch, West Grove, PA). After 5 to 7 days incubation at 4°C, tissue was again washed many times in WS over 2 days (Fig. 1e). (For more details about the tissue collection and processing methods employed here, see Woolley *et al.*, 2011.)

2.3. Tissue clearing and mounting

Control brain tissue slices and slices containing captured microelectrode arrays were optically cleared in a sucrose clearing solutions (Tsai *et al.*, 2009). A stock solution of 2% (v/v) Triton X-100 in HBHS was first made; sucrose was added to create concentrations of 0%, 15%, 30%, 45%, and 60% (w/v) sucrose in 2% TritonX-100 HBHS. Each tissue slice was incubated separately and sequentially from 0% to 60% sucrose with >4 hour intervals between each solution change. The above technique was performed in order to minimize tissue shrinking that can occur during tissue clearing, with changes in osmolarity (Tuchin,

2005). Tissue slices in the 60% sucrose solution were mounted in custom two-sided microscope slides (see supplemental material in Woolley *et al.*, 2011). Slices were stored in the dark at 4°C until imaging.

2.4. Tissue and device imaging

Imaging data presented in this article were gathered using a Zeiss LSM 710 inverted laser scanning confocal microscope (LSCM) equipped with a 2-axis motorized stage and 405, 488, 563, and 633 nm laser lines. Zen 2010 software (Carl Zeiss Microscopy, GmbH) was used to collect, stitch, and orient the imaging data. Cell quantification was performed using Cell Counter in ImageJ (NIH, Washington, D.C.). Figures were laid-out in Photoshop CS2 (Adobe Systems Inc., USA).

3. Results

3.1. Sucrose clearing solution extends fluorescent label collection depths

The optical refractive index modification properties of a high-concentration sucrose solution were confirmed on rat brain tissue slices of approximately 450 µm in thickness. Labeled with primary antibody to the microglia/macrophage-specific protein Iba1 and a secondary antibody excitable by 488 nm light, the depth penetration of a typical laser confocal z-stack was performed first in PBS, then in clearing solution (Fig. 1f). An imaging depth-limitation of approximately 200 µm was observed in PBS. In clearing solution this imaging was extended to the full 450 µm thickness of the slice. In both solutions PMT sensitivity and laser power were ramped in software during z-stack image collection.

3.2. Imaging microglia interactions at device/tissue interface

Brain slices containing 4-week implanted MEA shanks were labeled for Iba1, cleared in sucrose solution, and imaged under LSCM at points along the device interface. Sheaths of Iba1+ tissue were evident along the devices (Fig. 2b), which itself was imaged by collecting laser light reflectance from the device surface (Fig. 2a). The Iba1 signal formed a highly complex, non-uniform structure roughly coating and outlining the 4-week implanted shanks. Iba1+ filopodia appear to have been fixed while exploring the 3D surface of devices, including the electrode sites and the subtle depressions between polysilicon electrical traces, which raise the overlying device surface approximately 30 nm (Fig. 2c). Also of note were tight clusters of cells immediately surrounding the devices in superficial layers of the cortex (Fig. 2d). A light ‘clouding’ of Iba1+ signal was observed between the dark cell bodies composing these masses, which otherwise lacked an Iba1 signal. These masses were present in superficial cortex proximal to each device across subjects, with some masses extending wider away from the device, though none extending deeper than cortical layer II (Supp. Fig. 1).

3.3. Collection of device-length imaging panoramas

To further examine depth-related responses around the length of *in situ* devices in a consistent manner, tiled z-stack panoramas of fluorescent markers and device reflectance were taken using a motorized LSCM stage. Figure 3a presents a 3D block of Iba+ signal, composed of a 3–7 array of LSCM z-stacks collected over an implanted device using a 40x

objective. Stepping through individual layers of these data blocks allowed examination of the device interface and surrounding tissue in great detail (Fig. 3b). Digital adjustment of the pitch of the viewing plane in each 3D stack was then performed in Zen 2010 software; the resulting data sets closely match the angle of the device (Fig. 3c), and were used to visually interpret the intact device/tissue interface data. Maximum intensity projections of the desired thickness could then be generated at a pitch that closely matched the plane of the implant (Fig. 4). While the PMT voltage and laser power intensity were ramped during data collection to correct for signal loss with imaging depth into cleared tissue, fluorescent intensities was not typically even across the pitch-adjusted images due primarily to imaging depth.

3.4. Microglia variability with depth

Viewing the microglia/macrophage-specific marker Iba1 along the captured devices further revealed a complex depth-related response to the implants (Fig. 4). The anomalous dark mass was seen to contain low expression of Iba1 surrounded each implant in superficial cortical layers (Figs. 4a,b). A count of distinguishable Iba1+ cells showed a general decrease in cell numbers with depth, particularly after the first 400 μm of rodent cerebral cortex (Figs. 4c,d).

Amoeboid Iba1+ cells and Iba1+ cells with hypertrophic cytoplasm outlined the periphery of the dense tissue mass around the implant in superficial cortex (Fig. 5a). Hypertrophic Iba1+ cells were visible extending between 100 and 200 μm from the surface of the tissue mass in layers I/II, and although these cells were spaced apart they appeared more densely packed than is typical (Fig. 4b, d). Finely branched, ramified microglia, characteristic of resting state microglia (Streit and Xue, 2009) were present at approximately 100 to 200 μm from the dense cell mass.

Below the layers I/II centered mass, Iba1 expression was heavily present immediately surrounding microdevices (Fig. 5b). Iba1+ structures appeared morphologically similar to amoeboid monocytes grown in 2D culture, with a smooth Iba+ signal flattened along the 4 week implants. This signal appeared as semi-distinguishable cells and broken sheaths of likely multinucleated cells. The small troughs between polysilicon traces were well highlighted by Iba1+ signal at this cortical depth and device width. Ramified and morphologically 'resting' microglia were present within approximately 50 μm of the Iba1+ sheaths.

Iba1 signal was present further down the implant, in layers III/IV, with numerous cells clustered as amoeboid cells against the devices (Fig. 5c). These Iba1+ cells coated the surface, but were more distinct as individual cells compared with the smooth sheathing of Iba1+ signal visible more superficially around a slightly wider portion of the devices (Fig. 5b). Some Iba1+ cells with hypertrophic cytoplasm were present within approximately 10 μm of the amoeboid cells, while ramified, resting microglia were again present within 50 μm of the amoeboid Iba1+ cells. In cortical layers V/VI a somewhat attenuated Iba1+ tissue response was seen (Fig. 5d), however it appeared quite variable, with sporadic areas of heavy Iba1+ cellular masses. An obvious source of this variability was not seen. In some areas ramified resting microglia with thin processes were visible 10 to 20 μm from the

device surface, though the devices always had a mild to moderate coating of Iba1+ material and a few amoeboid and hypertrophic Iba1+ cells within approximately 20 μ m of the device.

3.5. Masses of cell nuclei around superficial device surface

In order to help determine the composition of the large masses surrounding our 4 week implanted MEAs in superficial cortex, the nuclei marker Hoe342 was imaged (Fig. 6). Each superficial cellular mass was brightly labeled by Hoe342 (Fig. 6b), with numerous tightly packed cell nuclei visible under 10x and 40x objectives. This tight packing of cell nuclei is morphologically similar in appearance to the typical histological view of the pia (Supp. Figs. 2, 3), which has the appearance of a mat of closely associated cells and cell nuclei. These cells did not exhibit a strong Iba1+ signal (Fig. 6d), which is expressed highly in microglia and non-CNS macrophages. These cellular masses did not appear to extend greater than 500 μ m along the device into the brain 4 weeks post-surgery.

3.6. Superficial cell clusters are positive for vimentin

To help determine the origin of this tightly cellular mass, antibody labeling of the astrocyte expressed glial fibrillary acidic protein (GFAP) was performed. Labeling of GFAP was not observed in or around the cellular mass (Supp. Fig. 1a). As the location and morphology of the cell mass suggested infiltration by meningeal-fibroblasts, we applied an antibody labeling against vimentin, a structural protein highly expressed in fibroblasts but not as widely expressed in the CNS. Vimentin label was present in and around the highly nucleated mass in superficial brain tissue (Fig. 7). As vimentin may also be expressed in cells within blood vessels, vasculature was also labeled, revealing little correlation with the vimentin labeling. These results indicate a non-CNS source for the superficial mass, likely composed of meningeal cells infiltrating the CNS by growing down the device surface over time.

4. Discussion

In this paper we utilized an advanced histology method ('DCHist') to collect and image interfacing tissue around intracortical MEA brain implants in rat brain tissue. We describe a complex, non-uniform microglia/macrophage coating along the surface of silicon devices, which appears to diminish in intensity with cortical depth/MEA width. Cellular processes expressing Iba1 were imaged against device surfaces, appearing to explore 3D features such as the electrode sites and troughs between underlying electrical traces. Finally we describe an anomalous tissue mass along implants in superficial cortex. Based on location, cell morphology, a lack of both Iba1 and GFAP labeling, and the presence of vimentin labeling, we interpret this dense mass of cells to be of non-CNS origin, and likely originating from the meninges.

Previous histological studies of the biological interface around intracortical MEAs have described a two-phase response to implanted devices: an acute phase dominated primarily by microglia and macrophage activity near the implant, and a chronic phase dominated primarily by the formation of a device-encapsulating astrocytic scar and the loss of nearby neuron cell bodies through death and displacement (see Leach *et al.*, 2010, for a detailed literature review). The present study adds histological evidence that chronic intracortical

MEAs experience a variable microglial response along their implantation depth, with substantially more microglial activity in superficial layers of cortex. This depth-related response is likely primarily influenced by the tapering dimensions of commonly used MEA devices, and the relative distance to the position where the MEA crosses from meninges into CNS tissue. The present study also describes a compact mass of many cell nuclei dominating the biological interface around MEAs in superficial cortical layers four weeks post-surgery. Expression of Vimentin, a marker of mesenchymal cells such as fibroblasts, was noted in this tissue mass, while a low expression of the macrophage and microglia marker Iba1 was recorded. This aberrant tissue structure appears composed of non-CNS cells progressively extending from the meninges along the device into the neocortex. Infiltration by fibroblasts and macrophages along transcranial implants has been investigated as a mechanism of device failure in chronic MEA implants (Kim, *et al.* 2004), and this study provides further evidence that a progressive invasion of non-CNS cells contributes substantially to the chronic phase of the tissue response around intracortical MEAs.

The work presented in this paper builds upon a formalized method to capture, label and image MEAs in thick tissue slices, a method which aims to avoid the removal and disruption tissue structures closely associated with brain-implanted devices during histological processing (Woolley *et al.*, 2011). Laser confocal imaging through thick tissue can prove challenging due to light scattering; we describe overcoming this problem by utilizing an optical index-matching ‘clearing’ solution, effectively extended the imaging depth of our microscopy. Due to decreased light scattering in clearing solutions (Tuchin, 2005), detailed imaging of fluorescent markers was performed in thick tissue slices under LSCM. Tissue clearing solutions have a long history of use in imaging underlying biological structures (Cumley *et al.*, 1939), and have recently experienced a resurgence as a useful tool to investigate thick, intact tissue specimens via microscopy (Zucker, 2006; Dodt *et al.*, 2007; Fu and Tang, 2010; Clendenon *et al.*, 2011; Hama *et al.*, 2011). Though imaging depth is improved greatly with use of a clearing solution, the ramping of laser intensity and sensitivity of the microscope detectors also aids in producing highly detailed z-projections for quantification and morphological interpretation. However, it should be noted that static microscope and laser settings may instead be used to produce data sets in which quantification of fluorescent intensity may be produced.

After imaging the cleared tissue, the collected DCHist data presented a detailed view of a complex tissue response around MEAs in cortical tissue. The Iba1 signal imaged formed a non-uniform structure roughly outlining the 4-week implanted shanks, with morphologies very unlike the typically discrete microglia cells. Across the subjects examined in this study a reactive Iba1+ signal was seen to surround a tissue mass in superficial cortex that did not express significant Iba1, and was seen to generally decrease with intensity around the device with cortical-depth/decreased device size. This Iba1 signal also appeared to have a relatively constrained volume, concentrated around the devices and not typically extended more than approximately 30 um away, except around the tissue mass in superficial layers. In tissue beyond the superficial Iba1-mass, clumps and sheaths of Iba1+ tissue were observed (Fig 6b), appearing as multinucleated giant cells. Deeper layers contained more discernible Iba1+

cellular processes visible along device surfaces (Fig. 6c,d), however individual cells were still challenging to identify due to indistinct boundaries.

Microglia cells typically repel one another while surveying, and will transition to various activated morphologies in response to perturbation. These states appear to include forming the multinucleated giant cells more typically seen in non-CNS tissues (Fendrick *et al.*, 2007). As previously suggested by other researchers, blood vessels proximal to intracortical MEAs may have chronically disrupted blood brain barriers. This disruption may allow peripheral immune cells easy access into CNS tissue near implants (Winslow and Tresco, 2010). In the case of a blood brain barrier damaged by an implanted microdevice it is possible that activated microglia and non-CNS immune cells both contribute to the clumping and sheathing observed on and around the intact devices in this study.

Across the subjects examined in this study, reactive microglial/monocyte preference for certain structures or materials on the devices was not clear. With further adoption of DCHist or similar intact histological methods, questions concerning device materials, device geometry, etc., may be more closely investigated from the perspective of biological impact and device/tissue integration. Insight into questions regarding subject-to-subject variability may also be better understood with DCHist investigation of the complex, depth-related tissue response. For example, in this study the exposed shank and bonding pad of implanted devices were cut in an attempt to avoid confounding effects of variable micromotion around devices tethered to the rodent skull across subjects (Biran *et al.*, 2007; Thelin *et al.*, 2011); the impact of this phenomenon along the length of implants may be more clearly analyzed with applications of DCHist in future studies.

With depth into the cortex, fewer activated microglia were observed surrounding the implanted devices. The tapered size of the implants used in this study likely influenced this result. It is also likely that tissue changes resulting from the surgical craniotomy affected microglial activity and density in a depth-dependent manner. Independent of its sources, the resulting variable microglia activity at different depths along the implant highlights the value of collecting histological data along the length of implants, as opposed to solely in cross-section.

The presence of numerous activated, rod-like and amoeboid-like microglia can be a useful component of the tissue response following insult, as debris and damage must be cleared. However, layers of microglia/macrophage cells appear to form living films across the surface of implanted devices, as seen in Figure 5. The build-up and repopulation of these structures atop recording or stimulating electrode sites is likely a significant source of day to day variability. While secreting pro-inflammatory cytokines, these activated microglia likely further induce chronic signal-to-noise degradation by disrupting nearby neuron, oligodendrocyte, and astrocyte structures through the secretion of cytotoxic chemicals in what has been described as the ‘frustrated phagocytosis’ of the implanted device (Hanisch, 2002; Biran *et al.*, 2005). A possible solution to address this issue is the development of very small biocompatible devices which elicit a smaller frustrated foreign body response to their chronic presence (Kozai *et al.*, 2012).

A surprising result of this work was the consistent presence of a highly nucleated tissue mass centered in cerebral cortical layer 2 and extending up into layer 1, appearing to be tightly adherent around the devices. These cells were not positive for Iba1, or GFAP, but were Vim+, indicating that fibroblasts may be a component. This mass may be contiguous with the meninges, and formed as the surgically disrupted meninges attempts to grow back over the site of injury around the shank of implant material. In Figure 6 a relatively intact pia is present, and although as with other slices the mass does not clearly extend up to the pia, a thin layer of meningeal cells may be present. It is also possible that the mass of cell nuclei in layer 2 was also present in cortical layer 1, but is consistently lost at some stage of brain removal along with the majority of the meninges. In either case, we contend that this mass is likely of non-CNS origin, and appears morphologically similar in appearance to the pia surrounding the rodent brain (Supp. Figs. 2, 3). Whether this is a progressive response that continues to extend down the surface of devices was not investigated; histology around devices implanted for many months may help to determine whether these cellular masses progressively descend along implants, or are limited to the superficial layers.

The compact mass of cells found around implants in superficial cortex in this study may well play a significant role in the tissue response to intracortical MEAs. A recent study from our lab investigating the depth-dependency of microstimulation-induced behavioral responses found that, over time, the most superficial electrode sites along a linear microelectrode array required the greatest increase in stimulation current to effectively drive a behavioral response (Koivuniemi *et al.*, 2011). Detailed histological analysis of tissue around intact devices used for depth-depended behavioral studies may clarify this link. If cells in this mass are indeed of meningeal origin, as our vimentin label indicates, it is possible that growth of this non-CNS tissue along and around implanted single or multishank implants may also affect electrode positions over time. In either case, a clearer understanding of the impact of a ‘healthy’ meningeal response to both craniotomy and intracortical implant would likely be informative to the development of increasingly biologically neutral intracortical MEAs (Nunamaker *et al.*, 2011).

Antibody to vimentin protein is the nearest to a fibroblast-related cell type label commercially available, and has been previously utilized in studies of fibroblasts and CNS injury (Carbonell and Boya, 1988). However, cell culture studies have measured macrophages secreting vimentin (Mor-Vaknin, *et al.*, 2003). We state in this report that the cellular mass tightly surrounding the device is likely primarily composed of fibroblasts because of a presence of Vimentin labeling coupled with a lack of Iba1 labeling, its location relative to the meninges, and the knowledge that fibroblasts can be migratory. The likely alternative cell type would be monocyte/macrophage cells; however, Iba1 is expressed in these cells and antibody labeling for Iba1 appeared quite low within the cellular mass. Morphologically the highly compact tissue mass in superficial cortex surrounding the implants is similar to multinucleated giant cells formed by aggregating macrophages, so it is possible that multinucleated giant cells in the brain might express a lower than typical amount of Iba1 compared to individual macrophages and microglia. Development of more specific ‘fibroblast-only’ antibody labels is an active area of research (Goodpaster *et al.*

2008), and a pairing of collagen antibody labels along with more specific fibroblast markers would help to further elucidate the origins of the tissue mass in future studies.

We have estimated that our current implantation strategy, utilizing micromanipulators and vacuum tweezers, results in a speed of insertion of approximately 2 mm/sec when implanting the single-shank MEAs for this study. It is possible, though unlikely, that this implantation speed coupled with these relatively sharp, tapered devices dragged or slightly depressed meningeal tissue into the brain along the device, effectively seeding this area with meningeal cells. Future studies utilizing faster or slower implantation of single-shank devices may answer how much speed of insertion impacts the presence of non-CNS tissue at various depths along chronic implants, especially if they employ a histology strategy that avoids removing the implanted devices such as DCHist.

The research presented strongly indicates that tissue depth/layer can significantly impact the tissue response examined. This study also reports that the method of collecting and imaging an intact interface is indeed valuable, as it provided valuable evidence that microglia/macrophages form highly variable clumps of tissue along the device with some depth-dependency, and demonstrated that a dense clustering of cell (likely fibroblasts) grow into the neocortex along the devices. Future studies focused on quantification of the variability of these tissue responses across many subjects and time points may now be undertaken utilizing the valuable techniques employed here to further our understanding of these device-interface phenomena. Through the use of advanced histological quantification tools (Bjornsson et al., 2008; Roysam et al., 2008), we hope to closely analyze optically cleared DCHist tissue data around a variety of implanted device designs, coated devices, and various other conditions to further our collective understanding of the biological impact of intracortical interface technologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DCHist	Device-Capture Histology
MEAs	Microelectrode Arrays

LSCM	Laser Scanning Confocal Microscopy
Trans	Transmission light image
Reflect	Reflectance light image
Iba1	ionized calcium binding adaptor molecule 1 (antibody label)
Vim	vimentin (antibody label)
GFAP	glial fibrillary acidic protein (antibody label)
Hoe342	Hoechst 33342
HBHS	HEPES-buffered Hank's Solution
WS	Wash solution

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Figure 1.

Overview of tissue preparation. (a) Unmounted, commercially available MEAs from NeuroNexus Technologies were utilized. Close-up microscope image showing the 16 Iridium electrodes (red, laser reflectance) along the silicon shank (grey, transmittance). (b) A vibratome was used to section fixed tissue; the blade is visible at left, the brain hemisphere being sliced is in center. A MEA (*) can be seen in the tissue to be sectioned next. (c) A collected slice with implanted MEA (*) is shown in a 24-well plate. (d) Fluorescent Iba1 antibody was imaged down to a depth of approximately 200 μm using a 488 nm laser in PBS. An extended imaging depth is seen when a 60% sucrose clearing solution was utilized as the imaging medium. Stacks are shown with the z plane oriented vertically, with individual LSCM images of 210 μm².

Figure 2.

Detailed view of device interface in cleared brain tissue histology. **(a)** Laser reflectance is shown in a projection of approximately 35 μm of tissue; polysilicon traces stand out from the Silicon surface, while circular Iridium electrode sites are also visible. **(b)** Iba1+ labeling outlines much of the device in layered, multi-cell masses, while individual microglia are visible a short distance from the device. **(c)** An expanded overlay from the boxed areas in **(a)** and **(b)** shows Iba1+ tissue (cyan) outlining much of the device surface (magenta) and grouping in non-uniform ways. Open arrow ($\rightarrow$) points to electrode site with Iba1+ filopodia exploring its surface. Full arrow ($\rightarrow$) points to Iba1+ tissue tracing the ~ 30 nm troughs between polysilicon traces. **(d)** Single optical z-section in superficial cortical layer I showing a cellular mass along the device (center), presenting a low Iba1 signal despite being surrounded by hypertrophic Iba1+ cells; sides of device are outlined by blue lines.

Imaging the full device and viewing data in the plane of implantation. **(a)** Large, z-stack data sets were collected around implants using an x-y controllable LSCM stage. The microglia/macrophage marker Iba1 is again shown in this example, with the cortical surface at the top of the image. **(b)** An image at $\sim 150\ \mu\text{m}$ in the stack shows part of the interface between tissue and device. Inset image illustrates how this image is parallel with the confocal scanning plane. **(c)** Adjusting pitch and yaw of 2D slices through the data set allows one to match the angle of the implanted device. The inset illustration shows how the pitch was adjusted in order to produce the plane shown below. Scale bars are $200\ \mu\text{m}$.

Figure 4.

Examining depth-related microglia variability along a 4-week implanted device. (a) Reflectance along an implanted device is shown. (b) Iba1 marker is shown through a 60 μm highest intensity z-projection. Overlaid boxes in (b) are expanded in Figure 5. (c) Device and tissue are shown in Trans, with an overlay of markers indicating Iba1+ cell positions. Quantification of Iba1+ cells was performed in bins beginning at the cortical surface and extending in 400 μm increments depthwise. A control area >1mm away was similarly imaged and quantified over a 60 μm highest intensity z-projection, and the resulting comparison was graphed for this subject (d). Scale bars are 200 μm .

Figure 5.

Examining morphology of microglia along a device. **(a-d)** In data expanded from Figure 4b, Iba1 marker is shown through a 60um highest intensity z-projection; blue lines indicate inside boundary of reflectance image from device surface. **(a)** A tissue mass immediately surrounding the device in superficial cortex (layers I/II) is itself poorly labeled for Iba1, though surrounded by both amoeboid, rod-like and bushy hypertrophic Iba1+ cells. **(b)** An Iba1+ sheath is seen in layer III, likely composed of amoeboid monocytes flattening along the device surface. **(c)** A compact clustering of Iba1+ cells is seen along the device in layers IV/V, appearing less sheath-like. **(d)** In layers V/VI a variable but typically less severe microglia response was observed around devices, with morphologically 'resting' microglia in relatively close proximity. Scale bar is 50 μm .

Figure 6.

Aggregate of cell nuclei in superficial cerebral cortex layers surround 4-week implanted device. **(a)** Cell nuclei and **(b)** antibody to Iba1 are shown around an implant in this representative tissue slice. A dense mass of cell nuclei is visible along the device, primarily in layers I/II of cerebral cortex. **(c)** Low Iba1 signal around the nuclei mass indicates that monocyte-derived cells are likely not playing a large role in the cellular mass. Images **(a)** and **(b)** are highest intensity projections through approximately 100 μm of tissue, while image **(c)** is a projection through approximately 20 μm of tissue angled with the device surface (not shown).

Figure 7.

Tight cellular masses around device in superficial cortex contain vimentin labeling. (a) Detailed imaging around the highly cellular mass, co-labeled for vimentin (b), revealed a complex expression of that protein in and around the anomalous tissue mass. (c) Blood vessels seen in close association with the mass do not directly overlap the vimentin (Vim) label (seen in the overlay image d), indicating that the vimentin signal is not primarily composed of blood vessel endothelial cells. (e) Trans image showing device location as a shadow (seen with respect to the fluorescent labels in image f). Images are 50 μm highest intensity z-stack projections. Scale bars are 50 μm .