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Biocompatibility evaluation of a thermoplastic rubber for wireless telemetric intracranial pressure sensor coating

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Abstract

This study investigated the biocompatibility of the experimental thermoplastic rubber Arbomatrix™ that will be used as the protective coating on a novel intracranial pressure (ICP) sensor silicon chip. Arbomatrix™ was benchmarked against biocompatible commercial silicone rubber shunt tubing in the brain via a rat model with 60-day implant duration. A bare silicon chip was also implanted. The results showed similar cellular distribution in the brain-implant boundary and surrounding tissues. Quantitative analysis of neuron and glia density did not show significant difference between implants. Through histological and immunohistochemical evaluation we conclude that Arbomatrix™ is well tolerated by the brain. Due to its exceptional barrier properties Arbomatrix™ has already been shown to be an excellent protective coating for new ICP monitoring chip.

Keywords

Cerebral cortex; Brain implants; Thermoplastic rubber; Reactivity; Toxicity; Biocompatibility

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Disclosure/conflict of interest

The authors declare no conflict of interest.

1. Introduction

There is a great deal of interest in novel technologies for diagnostic and therapeutic procedures for neurological diseases. The response of brain tissue to various sensors/electrodes implanted into the brain has been investigated (Polikov et al., 2005; Winslow et al., 2010). Often the metal sensors and electrodes have protective coatings. Several coating materials have been evaluated both experimentally and clinically and the majority has been discarded (Babb and Kupfer, 1984; Collias and Manuelidis, 1957; Dymond et al., 1970; Stensaas and Stensaas, 1978). Several plastics, aluminum, gold, and platinum have been tested *in vitro* and *in vivo* (Ammar, 1984; Stensaas and Stensaas, 1978). Acute mechanical injuries related to implants and insertion mechanics have also been studied (Welkenhuysen et al., 2011). It should be emphasized that these sensors/electrodes are in direct contact with neurons and are not buffered by cerebrospinal fluid.

We have been developing a wireless intracranial pressure (ICP) sensor on a silicon chip. Attempts to protect the device with a silicone rubber coating failed as water penetrating the coating caused a shift in the electric signal. Preliminary tests with Arbomatrix™, an experimental thermoplastic rubber based on polyisobutylene, have been very promising, due to the exceptional barrier properties of this material. Other advantages include ease of handling: the sensors are coated with Arbomatrix™ dissolved in a solvent, which will then solidify into a transparent rubbery coating without the need of curing. The biocompatibility and biostability of Arbomatrix™ in subcutaneous and intramuscular implantation in a rabbit model has already been demonstrated; its mechanical properties actually improved following explantation, while the benchmark silicone rubber lost some of its strength (Puskas et al., 2005). In order to use this material in the brain, its biocompatibility and biostability needed to be evaluated by implantation in the brain. As a part of our wireless ICP sensor project we histologically evaluated the biocompatibility of bare silicon and Arbomatrix™ after implantation in the cerebral cortex, benchmarked against biocompatible silicone rubber. The data from this study is presented in this manuscript.

2. Materials and methods

2.1. Evaluated materials

Sensor material (silicon chip, used for making microelectronic sensors), **Beads** (Arbomatrix™, a block copolymer of polyisobutylene and polystyrene with a branched polyisobutylene core, used for sensor packaging and coating) (Puskas and Chen, 2004; Puskas et al., 2005; Teck Lim et al., 2013), and **Control** (shunting tubing end, silicone rubber, Medtronic #41101, Goleta, CA) were used for implantation (**Fig. 2 A-C**). The **Beads** were cut from compression molded sheets of Arbomatrix™. The specimen sizes are given in the figure caption. The **Beads** were analyzed quantitatively before and after implantation by using Size Exclusion Chromatography (SEC) for possible degradation after implantation. SEC measurements were conducted at The University of Akron. The SEC system consists of an HPLC pump (Waters 515 HPLC Pump), a Waters 2487 Dual Absorbance UV Detector (UV), a Wyatt OPTILAB DSP Interferometric Refractometer (RI), a Wyatt DAWN EOS multiangle light scattering (LS) detector, a Wyatt ViscoStar viscometer, a Wyatt QELS quasi-elastic LS instrument (QELS), a Waters 717plus autosampler, and 6 Styragel VR

columns (HR6, HR5, HR4, HR3, HR1, and H0.5). The columns were thermostated at 35°C and THF, continuously distilled from CaH₂, was used as the mobile phase at a flow rate of 1 ml/min. The results were analyzed by using the ASTRA software (Wyatt Technology).

2.2. Animal surgery

Animal experiments were carried out in accordance with the Cleveland Clinic Animal Research Committee's rules for the care and use of laboratory animals (IACUC protocol # 2011-0646). Fifteen adult healthy male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 380-450 g were randomly divided into three groups for implantation: **Sensor**, **Bead**, and **Control**. Rats were housed in individual cages and maintained on a 12-h light-dark cycle with food and water available as required. All efforts were made to minimize the number of animals used and minimize suffering.

Animals were anesthetized with ketamine (80 mg/kg, intraperitoneal) and xylazine (5 mg/kg) and placed in the Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA). After the implants had been sterilized via ethylene oxide (EtO), they were inserted into the frontoparietal cortex through burr holes in the skull at the coordinate of AP: -2.0 mm; ML: 2.0 mm from bregma, and DV: under cortex (Paxinos and Watson, 2007). Routing post-operative cares include pain relieve with buprenorphine, neurological status evaluation, and monitoring for abnormal behavioral.

2.3. Sacrifice and sample collection for histological study

Sixty days post-implantation, rats were deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneal) in combination with inhaled isoflurane and underwent transcardiac perfusion with 0.9% saline followed by 4% paraformaldehyde (PFA) in 0.1M Phosphate Buffered Saline (PBS). Bulk brain tissues with the implants were carefully removed without damaging the brain-implant boundary. Brains were post fixed overnight and cryoprotected in graded sucrose solution for 24 h. After photographic evaluation of implant location, brains were blocked and frozen in optimal cutting temperature gel (OCT, Sakura Finetek, Torrance, CA) and stored at -80°C. Brains were later serially sectioned in the coronal plane at 20 µm using a cryostat (Leica Microsystems, Nussloch, Germany). Implants of **Bead** and **Control** were left in place and sectioned together with the brain tissues. Implants of **Sensor** (silicon chips) were carefully removed from the brain tissues before freezing and the cavities left by the removed **Sensor** were filled with OCT to keep an intact brain-implant boundary for cutting. The sections were thaw-mounted onto pre-cleaned superfrost plus micro slides.

2.4. Histological and immunohistochemical analysis

Hematoxylin and Eosin (H&E) staining was used to evaluate the implant location, inflammation, and general histological and architectural changes. Immunohistochemical (IHC) staining with glial fibrillary acidic protein (GFAP) was used to evaluate glial reaction (gliosis) (Eng, 1985; Polikov et al., 2005). Neuronal Nuclei (NeuN) IHC staining was used to evaluate possible neuronal toxicity of the implanted materials (Polikov et al., 2005).

H&E staining was followed by the standard protocol (Fischer et al., 2008). In brief, the slides were dipped in Hematoxylin (Gill 3, Fisher Scientific, Pittsburgh, PA) 10 times and

rinsed with tap water for 2 minutes, followed by dipping in acid ethanol 10 times. After rinsing in water, the slides were dipped in Eosin-Y (Alcoholic, Fisher Scientific, Pittsburgh, PA) 3 times and coverslipped with Cytoseal-60 mounting medium (Richard-Allan Scientific, Kalamazoo, MI).

Immunohistochemical staining for GFAP and NeuN were stained with Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. After rinsing with PBS, sections were incubated in 0.002% proteinase K solution for 20 minutes (GFAP) or 5 minutes (NeuN) at 37°C, followed by incubation in 3% H₂O₂ in methanol for 10 minutes. Tissue sections were then blocked with blocking serum for 1 hour. Primary antibodies were diluted in blocking solution, including mouse anti-GFAP (diluted in 1:200, #3670, Cell Signaling Technology, Inc., Danvers, MA) and mouse anti-NeuN (diluted in 1:200, MAB377, Millipore, Temecula, CA), and applied to sections in a humid chamber on a shaker overnight at 4°C. Negative controls were performed by applying blocking solution alone to different sections on the same slide. Sections were washed with PBS before diluted biotinylated secondary horse anti-mouse antibodies were applied for 30 minutes at room temperature. After washing, sections were incubated with avidin-HRP (ABC mix of VECTASTAIN Elite ABC Reagent) for 1 hour at room temperature. Immunohistochemical staining was visualized with 3'3-diaminobenzidine (DAB) mixed solution (DAB Substrate Kit, Vector Laboratories, Burlingame, CA) for 2-5 minutes. Counterstaining for GFAP was stained with Vector Methyl Green Solution (Vector Laboratories, Burlingame, CA). Finally, all slides were coverslipped with Cytoseal-60 mounting medium (Richard-Allan Scientific, Kalamazoo, MI).

2.5. Stereological counting methods

The density of neuronal and glial cells was estimated using a stereological cell counting method adapted from previous studies (Dombrowski et al., 2008; Dombrowski et al., 2001; West, 1993). An unbiased estimation of the numerical density of neurons and glia in the cortex is made by selecting three random fields adjacent to the implants from each animal. Total 90 fields were counted for neurons (NeuN+) and glia (GFAP+) from three groups: **Sensor** (n=5), **Bead** (n=5), and **Control** (n=5). Neurons and glia were counted using an optical dissector at 40 x magnification with a dissector volume of 1,276,000 μm^3 /field (height = 220 μm , width = 290 μm , thickness = 20 μm). Cell counting was performed under a Leica light microscope with a camera attached to a computer using the imaging capture program (Stereo Investigator version 5.04.5, Micro-BrightField, Williston, VT, USA), and mean cell numbers were calculated and statistically analyzed.

2.6. Statistical Analysis

Three cerebral fields from each animal were selected for neuron and glial cell counting respectively, and the mean values ($\pm$ standard error of the mean; SEM) of cell number from each animal were calculated. The cell numbers from three groups were analyzed by ANOVA (one way analysis of variance). If ANOVA yielded a significance of 0.05, subsequent Student's t-test was used to assess the difference between different groups. All data was presented as mean $\pm$ s.e. and $p < 0.05$ was considered significant.

3. Results

3.1. SEC analysis of Beads before and after implantation

Representative SEC traces of **Beads** before and after implantation are shown in Figure 1. All detector signals (LS–light scattering, Viscosity, RI–refractive index, UV– ultraviolet) remain unchanged after explantation, demonstrating that no degradation occurred in the material.

3.2. Location of implants and architectural changes

Implants were located in frontoparietal areas ranging from the rim of cortex to the deep brain tissues. No edema/swelling, hematoma and/or mass effect, and additional changes related to mechanical trauma occurred in the brain tissue surrounding the implants (**Fig.2 D-I**). Changes observed in the boundary layer and brain tissue surrounding **Sensor** and **Bead** implants were based on comparison with the changes in the **Control** group (silicone rubber shunt tubing). The variability encountered at the brain-implant interface was attributable primarily to the different implants and partially to the mechanical procedures during implantation and sample preparation for sectioning. To minimize the effect of this variability on the following cellular numerical evaluation for histological and IHC analysis, only areas located at anatomically similar cerebral tissues that showed minimal interference were randomly selected and analyzed.

3.3. Histological changes in H&E staining

Apart from the mechanical changes related to the implants' placement, no additional changes occurred. No necrosis, infection or inflammation changes were observed in any tissues of the three implant groups. There was no appreciable connective tissue surrounding the implants in the brains in all three groups. There were no evident difference among the three implant groups in the cellular constituents and distribution in the boundary layer in direct contact with the implant and the tissue surrounding the brain-implant boundary layer (**Fig. 3**).

3.4. Glial reaction

GFAP-IHC results showed condensed zones in the boundary layer between brain tissue and implants. Although the width and complexity of these zones varied, the same basic pattern was typical of intact tissues around all implants. The results showed similar glial distribution in the boundary layer and surrounding brain tissues and there was no apparent gliosis observed. There was no significant difference in glial cell numbers in the brain-implant boundary and the surrounding tissues among groups of **Sensor**, **Beads** and **Control** (ANOVA, $p=0.97$) (**Fig. 4**).

3.5. Neuronal toxicity

To evaluate possible neuronal toxicity of implant effect, neuronal numbers were assessed through NeuN-IHC. The results showed no apparent changes in neuronal density in the boundary layer and the surrounding brain tissues in three groups. The result showed similar neuropile regarding neuronal distribution and cellular density among the three implant

groups. The quantitative analysis of neuronal number showed no significant differences among **Sensor**, **Bead**, and **Control** groups (ANOVA, $p=0.179$) (**Fig. 5**).

4. Discussion

Precise histopathological determination of biocompatibility or toxicity of materials implanted in the brain is an essential step for evaluation of novel neurological devices for diagnostic or therapeutic procedures. As a part of the project, in this study, we investigated the effect of implanted materials, i.e., Arbomatrix™, an experimental thermoplastic rubber (**Beads**), shunting tube (silicone rubber, **Control**) and silicon chip **Sensor** material (widely used for making implantable, microelectronic sensors), after 60-day implantation in brain. Preliminary experiments showed that Arbomatrix™ could serve as an excellent rubbery barrier coating/encapsulating material for our novel ICP sensors. The results of histological and immunohistochemical evaluation with H&E, GFAP, and NeuN-IHC staining showed no observable side-effects related to histopathological reactions of the implanted materials.

Detection of reactive cellular changes depends on observations of the actual brain-implant boundary and surrounding tissues. Primary importance is maintaining an intact brain-implant boundary layer for evaluation. Loss of portions of the boundary layer adhering to the implant during its removal from the brain for histological procedures prevents precise examination of interface tissues (Stensaas and Stensaas, 1978). To avoid this problem, we left the rubbery implants (**Beads** and **Control**) in place during all stages of tissue preparation, and carefully removed the rigid silicon chips (**Sensor**) and filled the cavities left behind with OCT before freezing the specimens to keep an intact brain-implant boundary layer and surrounding tissues. This permitted a detailed evaluation of reactive cellular changes at the interface and the surrounding tissues.

To evaluate the changes definitely related to implant reaction, the observed changes must be distinguishable from those caused by mechanical trauma. Variability in the characteristics of the boundary layer surrounding a given implant was typical of the pattern of response to the implanted materials. This was apparently the result of mechanical disruption of brain tissue, which was attributable primarily to the different implants (soft and flexible rubbers and rigid silicon chips) and, to a lesser extent, to mechanical procedures during implantation and sample preparation for sectioning. To minimize the variability due to these effects, we evaluated the reactivity and biocompatibility of each material by investigating regions located at anatomically similar cerebral tissues that seemed to suffer minimal interference. Areas from these regions were randomly selected at more than one level in individual specimens, and at sections from several implants of the same material for further cellular numeric counting and analysis. Coordinated use of section series from the same block of tissue with different staining allowed consistent and positive identification and comparison of cellular constituents.

Earlier studies of the effects of enameled stainless steel electrodes implanted into cat brains showed the formation of a cellular capsule, loose connective tissue, and a dense zone of astrocytes (Collias and Manuelidis, 1957). However, it is unclear whether these changes were caused by trauma or by the materials used. In later studies bare metals such as silver,

copper, and nichrome alloy were compared with plastic-coated versions, such as polyethylene, polypropylene, and Teflon, to evaluate the cellular reactive changes. The results showed few changes near implants coated with polyethylene, polypropylene and Teflon (Bickford et al., 1957), and no sheath formation and little gliosis in the tissues surrounding small in-dwelling plastic (araldite) needles (Stensaas and Stensaas, 1976), but “noticeable damage and necrosis” in the vicinity of bare silver and copper (Baleyrier and Quoex, 1975; Bickford et al., 1957; Stensaas and Stensaas, 1978; Wilder et al., 1972). In the current study, the results showed similar cellular distribution and cell density in the brain-implant boundary and surrounding tissues in H&E staining, suggesting that no implant-related inflammation and cellular reaction occurred.

Traditional definitions of astrocyte activation and gliosis are based on morphology, numbers, and increases in GFAP expression (Eng, 1985; Fitch and Silver, 2008). Previous studies have demonstrated a varied degree of glial reaction in different time points after implanting of silicon in the brain (Polikov et al., 2005). In contrast, other groups showed no apparent difference of glial reaction between the implant groups of uncoated planar silicon microelectrode and the Parylene-C-coated silicon microelectrode (Winslow et al., 2010). In our GFAP-IHC staining, the results showed similar glial distribution and cell density in the boundary layer and surrounding brain tissues in the three implant groups: **Sensor** (silicon chip), **Bead** (experimental Arbomatrix™ thermoplastic rubber), and **Control** (silicone rubber shunting tube). Similar to the previous study (Stensaas and Stensaas, 1978), the brain-implant boundary layer was composed of a thin condensed zone that was similar to the glia-limitans that normally form the pial surface of the brain. In NeuN-IHC staining, in addition to the similar neuronal density and distribution in the areas of brain-implant boundary layer and surrounding brain tissues, no significant difference was found in neuronal numbers between our implanted materials, indicating no implant-related neuronal toxicity.

5. Conclusion

In summary, the implanted materials did not elicit inflammation, trauma, gliosis, and neuronal toxicity, resulting in normal-appearing cytoarchitecture. The implants were surrounded by apparently normal gray matter, indicating that displacement of tissue during surgical introduction left the neuropile and vasculature intact. Therefore we conclude that the central nervous tissue tolerated direct contact with the implanted “foreign” materials. We plan to use silicon chips coated with Arbomatrix™ for our wireless pressure sensor. Based on our results Arbomatrix™ coating could be used on other silicon-chip based devices.

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Figure 1. Size Exclusion Chromatography (SEC) traces of **Beads** before and after implantation. The molecular weight and molecular weight distribution of beads before and after implantation are shown and no degradation was observed in the implanted materials.

Figure 2. Implants and locations. Silicon chip (A, **Sensor**, rectangular, $2.95 \times 0.96 \times 0.38$ mm), Arbomatrix™ thermoplastic rubber (B, **Bead**, coating material, oval cross-section, $1.67 \times 0.77 \times 0.73$ mm), and shunting tube end (C, **Control**, silicone rubber, D = 1.62 mm, long side 1.90 mm, short side 1.35 mm) were located in right frontoparietal areas reaching from the rim of cortex to the deep brain tissues (D-I).

Figure 3.

Outline of the brain-implant boundary and the cytoarchitecture in H&E staining. The variability of brain-implant outline was attributable to the different implants and mechanical procedures during implantation and sample preparation. However in the relative minimal interfered regions, the cellular constituents and distribution showed similar cytological characteristics in the three groups.

Figure 4. GFAP-IHC staining. A thin capsule is visible in the boundary of brain-implant in all three groups. However there was no significant difference in glial distribution and cell numbers among the three groups ($p=0.97$).

Figure 5. NeuN-IHC staining. The result showed similar neuropile regarding neuronal distribution and cellular density in the three groups. The quantitative analysis of neuronal number showed no significant difference among the three groups ($p=0.179$).