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The foreign body response and morphometric changes associated with mesh-style peripheral nerve cuffs

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ABSTRACT

Nerve cuffs have been used to anchor and protect penetrating electrodes in peripheral nerves and have been used as non-penetrating electrodes for neural recording and nerve stimulation. The material of choice for such applications is silicone, an inert synthetic biomaterial which elicits a minimal chronic foreign body response (FBR). While histological studies of solid silicone cuffs are available, to the best of our knowledge a comparison to other cuff designs is not well documented. Here, we describe the FBR and morphological changes that accompany nerve cuff implantation in the rat sciatic nerve by comparing a metallic mesh with and without a parylene coating to one made of silicone. Two months after implantation, we observed that such implants, irrespective of the cuff type, were associated with a persistent inflammatory response consisting of activated macrophages attached to the implant surfaces, which extended into the endoneurial space of the encapsulated nerve. We also observed foreign body giant cells in the epineurial space that were more prevalent in the mesh cohorts. The mesh cuff groups showed significant changes in several morphometric parameters that were not seen in the silicon group including reductions in nerve fiber packing density and a greater reduction of large diameter fibers. High magnification microscopy also showed greater evidence of foamy macrophages in the endoneurial space of the mesh implanted cohorts. Although the precise mechanisms are unknown, the results showed that mesh style nerve cuffs show a greater inflammatory response and had greater reductions in morphometric changes in the underlying nerve compared to silicone in the absence of a penetrating injury.

Statement of Significance

While traditional silicone cuffs have been in use for decades, the inflammatory and morphometric effects of these cuffs on the underlying nerve have not been deeply studied. Further, manipulation of the foreign body response to nerve cuffs by using various materials and/or designs has not been well reported. Therefore, we report the inflammatory response around nerve cuffs of various materials and designs, as well as report morphometric parameters of the underlying nerve. These data provide important information regarding the potential for quantitative morphometric changes associated with the use of nerve cuffs, and, importantly, suggests that these changes are associated with the degree of inflammation associated with the cuff.

1. Introduction

Microelectrode arrays implanted into the peripheral nervous system (PNS) offer the potential to record from and stimulate nervous tissue for use in the control of neuroprosthetic devices. Such arrays vary in design and include non-penetrating, multi-lead cuff electrodes, sieve or regenerating electrodes, and other penetrating multi-lead or multi-shank designs. These devices are used clinically in respiratory pacing [1–3], as sacral root stimulators for urinary and fecal management [4–9], and as peroneal nerve stimulators for the treatment of foot-drop [10–12].

Cuff style, non-penetrating electrodes in particular have been in use since the late 1940s when Whittenberg et al. stimulated the phrenic nerve to assist in diaphragmatic pacing [13]. The foreign body response (FBR) surrounding such synthetic cuff electrodes in PNS applications has been relatively well studied [1,14–21]. While the implantation of a cuff electrode does not cause a penetrating injury to the underlying nerve, their chronic use has been

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associated with alterations in nerve composition, including changes in fiber density [17,22,23], fiber diameter and g-ratio (ratio of axon diameter to total fiber diameter) distribution [16,23,24], the degree of connective tissue deposition [1,10,15–20,23,25,26], and the infiltration of macrophages into both the epineurial [1,19,20] and endoneurial [1,20] space. While the biological mechanisms behind such changes remain unclear, various cuff designs have been shown to increase pro-inflammatory cytokine levels [19,20] and enhance blood-nerve-barrier (BNB) permeability [27], so it is possible that the persistent macrophage activation that drives the FBR may underlie the morphometric changes observed with certain types of cuff use.

In addition to their use as electrodes, nerve cuffs recently have been used to anchor and protect penetrating electrodes at the site of implantation [28–37]. Changes in nerve composition due to the use of cuffs have been associated with these applications as well [28,29]. Several studies also have shown that the composition of the cuff does not alter many aspects of the foreign body response [24–26].

It has been suggested that the use of solid cuffs may exacerbate FBR sequela by causing compression of the nerve following overgrowth of encapsulation tissue located between the inside of the cuff and the nerve [38]. It also is possible that changes result from continuous macrophage activation over the indwelling period or the mechanical mismatch between the cuff and the encapsulated nerve segment. At present it is unclear whether the use of different cuff types, such as open mesh cuffs which could potentially mitigate issues related to connective tissue overgrowth, or those made of different materials differentially affect the FBR or are associated with morphometric changes to the underlying nerve segment. This is particularly important with regard to cuffs or encapsulating materials used to anchor penetrating electrodes where different materials and mesh style cuffs made of gold have been used for their electrical shielding properties [39]. To begin to address this knowledge gap, we compared the FBR to an open mesh metallic design with and without a coating of parylene-C to one made of silicone using the rat sciatic nerve model, and asked whether the use of such cuffs was associated with morphometric changes in the encapsulated nerve segment [40,41].

2. Methods

2.1. Cuff implantation

Studies were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Utah. Three groups of adult male Fischer 344 rats (200–225 g, N = 8 each group) were implanted, each with either a solid silicon cuff (Kwik-Cast, WPI, Sarasota, FL), a size 52 gold mesh cuff (Alfa Aesar, Ward Hill, MA), or a size 52 gold mesh cuff coated with Parylene-C (~5.5 μm coating thickness). A Parylene-C coated cohort was included based on previous literature showing decreased inflammatory cell attachment to Parylene-C coated surfaces [42,43]. All cuffs were of equivalent size of 6 mm in length. For implantation, animals were anesthetized, the leg was shaved and treated with betadyne, an incision was made from the hip to the knee, and the biceps femoris and vastus lateralis muscles were separated to expose the sciatic nerve. For the gold mesh cuff and the Parylene-C treated cohorts, meshes 5.5 × 6.0 mm in length, which were previously sterilized with ethylene oxide gas exposure (University of Utah Medical School core facility) were loosely wrapped around the nerve until the sides of the cuff were slightly overlapping. The sides were then tied together using surgeon knots with Ethicon Vicryl Plus 6-0 degradable sutures (esutures.com, Mokena, IL). Prior to implantation, the ends of the gold meshes were dipped in silicone to prevent any tissue damage from sharp ends. For the silicone cuffs, the nerve was gently lifted from the underlying tissue and Kwik Cast Sealant (WPI, Sarasota FL) was applied according to the manufacturer's directions around the nerve for a distance of approximately 6 mm so that the nerve was encapsulated in a similar fashion to the gold mesh and as previously described [35,37]. The distal end of each cohort of nerve cuffs was located 1 cm proximal to the bifurcation of the tibial and common peroneal branches. Following cuff implantation, the muscles were sutured using Ethicon Vicryl Plus 5-0 degradable sutures and the skin was closed using Ethicon Ethilon 4-0 nylon sutures. A group of age-matched animals (N = 8) which did not undergo surgery were used as controls.

2.2. Euthanasia and tissue preparation

Approximately 2 months after implantation (60 days), animals were terminally anesthetized and transcardially perfused with pH 7.4 phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The time point was chosen based upon similar previously published studies [25,29–32,44]. The sciatic nerve from each leg was dissected free and post-fixed in 4% paraformaldehyde in PBS overnight and then stored in PBS with 0.01% sodium azide at 4 °C.

The post-fixed nerves were hand cut in cross-section immediately distal to the nerve cuffs to produce nerve segments approximately 2 mm in length. Synthetic cuffs were then carefully removed using microdissection scissors and processed for immunohistochemical labeling. Sections from age matched unimplanted animals were taken 1 cm proximal to the bifurcation to serve as controls for morphometric analysis [45]. Nerve segments were further post-fixed in 4% paraformaldehyde/2% glutaraldehyde in PBS for 24 h followed by equilibration in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for 24 h. Sections were then dehydrated through a graded series of ethanol, cleared with propylene oxide, and embedded in Embed 812 (Electron Microscopy Sciences, Hatfield, PA). Sections were cut at 0.6 μm using an Ultracut EMUC6 (Leica, Bannockburn, IL), placed on Superfrost Plus glass slides, and stained with thionin and acridine orange (Sigma-Aldrich, St. Louis, MO). Sections were coverslipped using Cytoseal XYL (Richard-Allan Scientific, Kalamazoo, MI).

2.3. FBR analysis

Explanted solid and mesh cuffs were analyzed for markers against CD68 to identify activated macrophages and foreign-body giant cells at the material-tissue interface. Solid and mesh cuffs were placed in wells and incubated in a blocking solution consisting of 4% (v/v) goat serum (Invitrogen, Carlsbad, CA), 0.05% (v/v) Triton-X 100, and 0.01% (w/v) sodium azide overnight at room temperature. Sections were then treated with primary antibodies against CD68 (0.5 μg/ml, AbD Serotec, Raleigh, NC) in blocking solution for 24 h and followed by three room temperature rinses in PBS (2 h/rinse). The appropriate secondary antibodies (2 μg/ml each, Invitrogen, Carlsbad, CA) in blocking solution were added for 24 h and followed by three additional room temperature rinses in PBS. Sections were counterstained with DAPI to visualize cell nuclei. Cuffs were then placed on slides and imaged using a FluoView 1000 confocal microscope (Olympus, Center Valley, PA).

2.4. Quantitative analysis

Thin section images were obtained using ImagePro Plus 4.0 (MediaCybernetics, Bethesda, MD) and a color CCD camera (Photometrics, Tucson, AZ) attached to a Nikon E600 microscope. Imaging and quantification were conducted as previously described [45]. Whole nerve images were obtained at 200× final

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