Histological Evaluation of Chronically Implanted Tissue-Engineered-Electronic-Neural-Interface (TEENI) Devices


Abstract—Neural-interface devices have the potential to isolate and transduce both afferent (sensory) and efferent (motor) neural signals of the peripheral nerve to and from electrical signals in instrumentation for stimulation and recording to produce fine control of advanced prosthetics. In order to potentiate the full spectrum of possible applications, the persistent foreign-body response needs to be addressed. Here we describe the cellular and extracellular components of chronically implanted polyimide threads suspended within a tricomponent hydrogel. The results of these experiments will contribute to design modifications for future fabrications of tissue-engineered-electronic-nerve-interface (TEENI) devices.

I. INTRODUCTION

Prosthetic device technology has advanced beyond simple motor movement driven by electromyographic control, towards complex cyber biomimicry rivaled only by nature. In order to realize the full potential of both movement and sensory perception within advanced prosthetic devices, several investigators have begun to explore electroneurographic signals of the peripheral nerves proximal to the site of amputation as a means for unprecedented fine motor control and sensory perception.

Solid-state microelectrode arrays implanted into the proximal nerve have shown promise in clinical settings, but both animal and clinical studies provided evidence that the inflammatory response and subsequent bioburden associated with the foreign-body response (FBR) of chronically implanted devices may significantly dampen the sensitivity and efficacy over time [1]. Alternatively, peripheral-nerve cuffs bearing electrodes may avoid impaling the epineurium, but the increase in distance from axons significantly reduces the strength of recordable signals and is further complicated by noise originating from the local muscle tissue [2].

The FBR has been well investigated following both acute and chronic implantation within the cortex [3]. Disruption of the blood brain barrier is initiated by device implantation and leads to a cascade of direct and paracrine inflammatory responses. The resulting glial scar causes significant encapsulation of the device and reduces the efficacy of recording local action potentials over time [4]. This classic FBR has led investigators to test various methods to reduce dual fibroblast invasion, glial scarring, and cellular reactivity through device surface modifications and coatings [5].

Peripheral-nerve interface devices attempt to incorporate electrodes into a regenerative environment that could provide a more stable and specific interface if the FBR can be contained. Unfortunately, little descriptive information is available within the literature making it difficult for design modifications. Furthermore, unlike the brain, the peripheral nerve contains a dense population of resident fibroblasts that are essential for successful regeneration. Therefore, techniques used to reduce the FBR in the CNS may not be optimum for peripheral-nerve-device designs.

Once the peripheral nerve is inflamed or severed, the distal portion will undergo a choreographed metamorphosis involving degeneration of myelin and affected axons, dedifferentiation of Schwann cells, infiltration of activated macrophages, and upregulation of vimentin expressing fibroblasts. The purpose is to phagocytize inhibitory debris and remodel the distal nerve to an environment more conducive for regeneration through deposition of newly synthesized extracellular matrix (ECM), release of degrading enzymes, and expression of trophic factors. With that in mind, affixing an acellular conduit with an embedded neural device suspended in a biodegradable hydrogel to the severed proximal stump, would incorporate the innate ability of the peripheral nerve to regenerate while avoiding confounding factors associated with local cellular reaction and Wallerian degeneration. Furthermore, this would allow an investigation of the FBR in a controlled environment that could lead to subsequent design modifications to improve peripheral-nerve-device functionality for years.

II. METHODS

A. TEENI Device Assembly

All assembly procedures were performed using aseptic techniques. The proposed first-generation TEENI device consists of microfabricated polyimide thread-sets suspended in a biodegradable hydrogel (laminin, collagen I, and methacrylated hyaluronic acid) wrapped in a decellularized small intestinal submucosa (SIS) (Figure 1). For preliminary

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FBR characterization, several polyimide threads (10 µm thick, 100 µm wide and 5 mm long) were freely suspended within the hydrogel to isolate the device from the surrounding muscle bed and external interstitial tissues. A sheet of SIS was wrapped around the suspended threads and hydrogel to form a tube structure with 4 interrupted sutures. This pre-TEENI device was used for all surgical procedures and histological evaluations in this report.

**B. Surgical Implantation**

All animals and procedures were reviewed and approved by the University of Florida Animal Care and Use Committee. Fifteen male Lewis Rats (250g, Charles River Laboratories, Kingston, NY) were used during this study and were maintained in accordance with the Guide for the Care and Use of Laboratory Animals.

Animals were induced with 3% isoflurane, clipped, and the surgical site aseptically prepared using alternating chlorhexidine and saline scrubs. A cutaneous incision was made over the lateral aspect of the right hind limb and the sciatic nerve was exposed through separation of the biceps femoris and vastus lateralis muscles. Once the main sciatic nerve was freed from the underlying fascia, a 4-mm-long segment was excised 4-mm distal to the ilioformal ligament and the pre-TEENI device was gathered in place to both the proximal and distal nerve stump using 2 interrupted 9-0 sutures. The muscles were approximated and sutured with 5-0 suture in a simple continuous pattern. The skin was closed with 5-0 suture in a subcuticular pattern. Rats received 4 mL of warm saline IP and were allowed to recover from anesthesia with thermal support. All rats received 1-2 mg/kg meloxicam once daily for up to 3 days following surgery and were maintained for up to 6 weeks.

**C. Immunohistochemical Analysis**

At the time point for tissue collection, rats were euthanized by deeply anesthetizing them with 3 to 5% isoflurane in oxygen followed by perfusion with PBS and 4% paraformaldehyde. Pre-TEENI devices were carefully dissected from the underlying muscle bed and processed for immunohistochemistry. Tissue samples were post fixed in 4% paraformaldehyde for 24 hours at 4°C, washed thrice in PBS, and cryoprotected in 30% sucrose buffer for 48 hours. Tissue samples were cut at mid device and embedded for cryosectioning. Frozen tissue sections were cut (20 µm thick), baked at 50°C for 20 min, and washed twice in PBS to remove mounting media. Non-specific binding was prevented by exposing tissue sections in a 10% normal goat serum in PBS (blocking buffer) for 1 hour prior to primary antibody incubation. Triple immunofluorescent techniques were employed by incubating tissue sections with a mixture of monoclonal and polyclonal primary antibodies simultaneously (Table 1). Prior to use of C6S and C4S antibodies, tissue sections were pretreated with chondroitinase ABC for 90 minutes at 37°C [6]. Pretreatment of tissue sections with proteinase K was required prior to vimentin and CD68 antibodies. Primary antibody cocktails were incubated on tissue sections overnight at 4°C. After several washes in PBS, bound primary antibodies were detected by indirect immunofluorescence using a mixture of secondary antibodies directed against mouse and rabbit or mouse and chicken conjugated to various fluorophores diluted in blocking buffer (Table 1). Tissue sections were then counterstained with DAPI (1µg/ml solution in PBS; Thermo Fisher Scientific, Massachusetts, USA) to visualize cellular nuclei. The resulting quadruple fluorescent and immunolabeled tissue sections were washed several times in PBS, rinsed in water, and cover slipped using an aqueous mounting medium containing anti-fading agents (Vectashield; California, USA). Digital images were captured at low and high magnification using oil emersion on a fluorescent microscope equipped with several excitation and emission channels (Axio Scope 2; Zeiss, California, USA).

**III. RESULTS AND DISCUSSION**

**A. Cellular Response to Chronically Implanted Pre-TEENI Device**

Pre-TEENI devices were sampled at mid-graft and subjected to extensive immunohistochemical analysis. The immediate cellular environment following implantation of a neural device will influence the FBR. The use of an acellular...
hydrogel graft allowed us to investigate the deposition of newly synthesized extracellular matrix associated with both the regenerative and FBR, while avoiding confounding factors associated with local cellular reaction and Wallerian degeneration.

All grafted segments were well tolerated and supported axon regeneration as indicated by the presence of axons and Schwann cells at mid-graft (Figure 2). Although DAPI signals indicated hyper-cellularity throughout the graft, Schwann cells only appeared to colocalize with regenerating axons. Regenerating axons and Schwann cells did approach the pre-TEENI polyimide threads, but did not appear to circumscribe each thread as hypothesized. Rather, the axons and Schwann cells appear to remain outside of the thread cluster.

![Fig. 2. Neuronal and non-neuronal cellular response to chronically implanted pre-TEENI device.](image)

Vimentin is a cytoskeletal intermediate filament commonly found in mesenchymal cells and fibroblasts. Immunoreactive profiles against the vimentin antibody were detected throughout the grafted material indicating the ubiquitous yet distinct presence of fibroblasts (Figure 3). In the tissue, immediately adjacent to the implanted threads, vimentin appeared as dense plaques. On the other hand, vimentin detection was loosely striated when associated with the tissue that contained axons and Schwann cells.

![Fig. 3. Foreign-body response to chronically implanted pre-TEENI device.](image)

Macrophages were detected around the polyimide threads using a monoclonal antibody against CD-68 (Figure 3). Interestingly, CD-68 immunoreactive cells were interspersed between the threads and virtually absent near regenerating axons. Additionally, CD-68 immunoreactivity did not colocalize with vimentin dense plaques but appeared to circumvent them.

![Fig. 4. Extracellular matrix deposition of chronically implanted pre-TEENI device.](image)

Isolectin B4 binds to terminal galactose residues found on the cell surface of stimulated macrophages and endothelial cells. Fluorescent profiles associated with iselectin B4 binding appeared as a thin boundary that isolated the vimentin dense plaques from the diffuse fibroblasts and macrophages of the interstitial tissue within the FBR of the threads (Figure 3).

B. Extracellular Matrix Deposition to Chronically Implanted Pre-TEENI Device

Laminin is a major component of the Schwann cell basal lamina and contains strong promoting properties on regenerating and sprouting axons. Laminin was present in the tissue area corresponding to axons and Schwann cells that circumvented the polyimide threads (Figure 4).

Chondroitinase ABC, is a bacterial lyase that degrades the sugar chains off the core protein of chondroitin sulfate proteoglycans revealing a neoepitope to which antibodies have been developed against. Chondroitin-4-sulfate (C4S) antibody detects the residual neoepitope if a sulfate is positioned on the 4-carbon while chondroitin-6-sulfate (C6S) will specifically bind if the sulfate is detected on the 6-carbon position. Following chondroitinase ABC treatment, C4S immunoreactive proteoglycans were found to be associated with think fibrous bands within the FBR of the threads (Figure 4). Meanwhile, C6S was isolated to the ECM of regenerating axons (Figure 5). This complementary distribution of chondroitin sulfate proteoglycan subtypes has
been described in both normal and regenerated peripheral nerve [6].

C. Characterization of the Foreign-Body Response

High magnification images of quadruple-fluorescent-labeled tissue surrounding the pre-TEENI threads indicate that there are at least 3 sub-populations of cell groups. Fibroblasts appear to form dense vimentin plaques closely associated with the polyimide threads, without the presence of CD68 immunoreactive macrophages (Figure 4). Immediately adjacent to the vimentin plaques appeared a thin boundary that bound strongly to isolectin B4. Meanwhile, the interstitial fibrous tissue surrounding the isolectin B4 reactive boundary was immunoreactive to the C4S antibody. Interestingly, these concentric layers of distinct ECM components correlated with the presence of fibroblasts alone, macrophages alone, and cohabitation of fibroblasts and macrophages respectively.

The tissue surrounding the FBR contained a much different cellular and ECM components (Figure 5). Schwann cells and vimentin immunoreactive fibroblasts were present throughout the tissue where axons were detected. Laminin was deposited in the newly formed Schwann cell basal lamina and appeared to colocalize with C6S immunoreactive proteoglycans. Although C4S proteoglycans were present outside the FBR, the spatial relationship relative to laminin and C6S proteoglycans appeared to complement rather than colocalize where axons and Schwann cells were present.

IV. CONCLUSIONS

This immunohistochemical analysis of the FBR of chronically implanted peripheral-nerve devices should establish a methodology for future experiments. Based on these results, efforts focusing on reducing inhibitory and encouraging permissive regenerative matrix deposition are underway.

V. REFERENCES