A Chronic Window Imaging Device for the Investigation of In Vivo Peripheral Nerves

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Abstract— **Chronic imaging of the peripheral nervous system with contemporary techniques requires repetitive surgical procedures to reopen an area of interest in order to see underlying biological processes over time. The recurrence of surgical openings on an animal increases trauma, stress, and risk of infection. Such effects can greatly lessen the physiological relevance of any data recorded in this manner. In order to bypass repetitive surgery, a Peripheral Nerve Window (PNW) device has been created for chronic *in vivo* imaging purposes. Intravital imaging window devices have been used previously to image parts of the rodent model such as the brain, spinal cord, and mammary tissue, but currently have not been used in the peripheral nervous system because of lack of bone anchoring and access to deep nerve tissue. We demonstrate a novel surgical technique in a rat which transposes the sciatic nerve above the surrounding muscle tissue allowing the PNW access to an 8mm section of the nerve. Subsequent days of observation revealed increased vasculature development primarily around the nerve, showing that this preparation can be used to image nerve tissue and surrounding vasculature for up to one week post-implantation.**

I. Introduction

In vivo or intravital imaging is an important tool that is utilized by a number of disciplines ranging from neuroscience and cancer biology to developmental and stem cell biology for the purpose of visualizing and documenting a working biological process in its most physiologically relevant context.

The technical challenges of adopting these monitoring technologies are great, but the extensive amount of information that can be collected from minimally disturbed properly functioning systems is an invaluable asset to researchers. Minimal perturbation to a biological system also yields data and results more conclusive to a natural biological process. Such monitoring allows understanding of a biological process not only in a relevant spatial and temporal context, which can be difficult to do with traditional ex vivo studies, but also can capture the nature of the biological microenvironment including signaling, chemical and physical factors which is currently not feasible to do with a purely in vitro system.

There have been many groups that have used windowed devices to gain access into parts of the body such as to chronically image mammary tissue [1,2], spinal cord [3,4], and neural cortex [5–7]. *In vivo* cranial window imaging has become popular recently because of its ease of implantation and powerful capabilities of being able to image directly into the brain in a chronic and even awake and behaving animal [8].

Currently there are few to no techniques for chronic imaging of peripheral nerves or nerve fibers that are not located superficially in mammals. Contemporary techniques are largely invasive due to the continuous perturbation of the biological system under observation. The current method remains to create an incision, image, and either sacrifice the animal or close the wound to reopen later for further testing [4]. This creates scar tissue, increased routes for infection, damage to the nerve due to over manipulation, and undue stress on the animal. Such effects can potentially impact any results obtained via this method.

There have not been any studies to date that have used a chronic imaging preparation in the peripheral nervous system (PNS), that do not involve invasive opening and closing of wounds [4], or that involve the limitations of imaging only superficial nerves [9,10]. There is great need to observe which biological processes are occurring during normal PNS function, or, more importantly, what occurs during pathological phenomenon [11]. In order to move past current practices and access deeper portions of the nervous system over time, which is currently unavailable over a period of time longer than one day, we propose a method that would safely and effectively raise the intact peripheral nerve into viewing range. This method includes the fabrication and implantation of a device, the Peripheral Nerve Window (PNW), which would allow for chronic imaging of the nerve in view.

II. Methods

A. Device Fabrication

The Peripheral Nerve Window device was created from three components: a cylindrical annulus, a tower-key locking system, and a 10mm diameter window (Fig. 1.). The cylindrical annulus was created using 3-D printing rapid

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prototyping technology from a (computer aided design) CAD-software-file [(StereoLithography) SDT, file format,

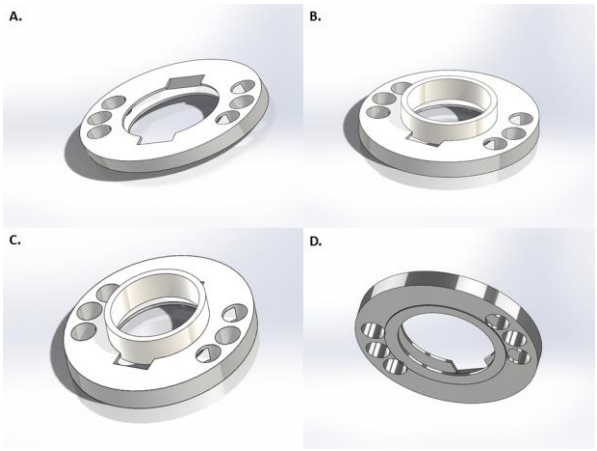

Fig. 1. Technical illustration of the PNW. Panel A displays the finished design of the base. Panels B & C show the inserted stainless steel piece into the base of the design. Panel D shows the underside of the base with attached window. Note the window is transparent and outlined on the bottom of the device in Panel D.

3D systems] created in SolidWorks (Dassault Systems). Within SolidWorks, the base for the interior implant was a 3mm thick cylinder with an outer diameter of 15mm and an inner diameter of 8mm. Further modifications were performed using extruding techniques in SolidWorks to create a key and lock mechanism within the device for the tower to be fitted within it. Two rectangular interior extrusions were placed on the top and bottom of the anterior face of the annulus to provide area for the tower-key to drop into the structure. Within a depth of 1mm, an additional 1mm extrusion and a 90° rotational extrusion was added to provide area for the tower-key to be placed. Fig. 1 shows the CAD file of the cylindrical annulus.

The file was uploaded onto the DimensionElite 1200es 3-D printer (Stratasys) using a 0.254 mm layer resolution. Biocompatible acrylonitrile butadiene styrene (ABS) was used as plastic resin for the creation of the part.

The tower-key was an additional stainless steel cylindrical annulus created with two protruding rectangular metal latches of 0.25mm uniform length, width and height. The material used for the tower-key was corrosive resistant 316-stainless steel. The tower-key was created on a Hardinge lathe and finished with an electronic dremmel rotary tool. The final component, the 10mm window was pre-fabricated and purchased from Harvard Apparatus.

B. Surgical Procedure

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin - Madison. All efforts were made to minimize animal discomfort. Animals were placed on a water-recirculating heat pad for temperature control and were monitored for vital signs every 15 minutes with a pulse oximeter and thermometer. After the induction of general anesthesia using 1.5-5% isoflurane in oxygen and an injection of buprenorphine hydrochloride (0.05 mg/kg, Reckitt Benckiser Healthcare Ltd), the hind limb of the rat was shaved and prepped using sterile technique.

The rat was placed in the prone position, and a 1cm skin incision was made along the dorsal aspect of the thigh overlying the hindlimb musculature. The skin margins were retracted and elevated in order to expose the underlying proximal dorsal hind limb musculature. The intersection between the biceps femoris and tensor fasciae latae muscles was carefully identified and gently dissected apart. This maneuver exposed the sciatic nerve located in the interval between the muscle edges. Gentle dissection was then carried out deep to the nerve in order to free it from the surrounding soft tissues. The intact and now mobile nerve was then elevated and a supero-lateral slip of the biceps femoris muscle was passed under the nerve in order to elevate it into a subcutaneous plane. This slip of muscle was anchored to the tensor fasciae latae edge with two horizontal mattress sutures using 5'0 silk. This provided an elevated and stable base for the transposed sciatic nerve to rest on (Fig. 2.).

After gentle transposition of the sciatic nerve, the windowed device was set in place over the nerve and sutured through holes in the ABS ring to the bottom of the skin with 5-0 polypropylene non-absorbable sutures. The remaining wound incision was stapled closed. A second dose of buprenorphine was given 8-12 hours after the initial dose for pain management and triple antibiotic ointment and ampicillin (50 mg/kg, Sage Pharmaceutical) were administered twice daily for one week post-surgery to prevent infection.

C. In Vivo Imaging

Chronic imaging was done on one rat for up to one week after device implantation. In each imaging session, the animal was anesthetized with dexmedetomidine(0.05mg/kg, Orion Pharma), and continuously supplemented with oxygen at a rate of 0.8 L/min. The animal was placed on a water-recirculating heat pad, and its vitals were monitored using a pulse oximeter. The animal was placed on its right lateral

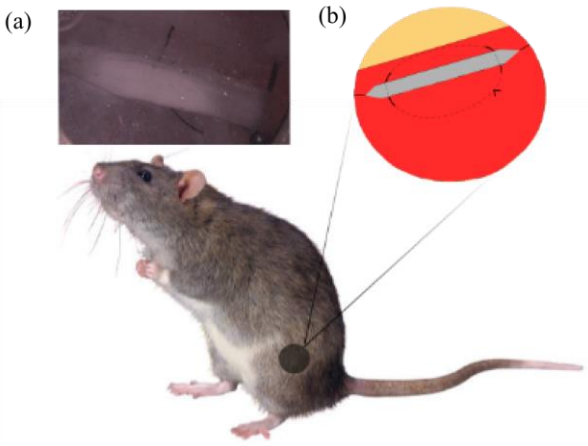

Fig. 2. Above, inset (b) illustrates the surgical procedure involving transposition of the sciatic nerve onto the biceps femoris into viewing

range, and inset (a) is a photograph of the surgical procedure of this method done in cadaver tissue.

side to expose the left lateral surgical implant. Upon placement, an upright fluorescent stereoscope (Leica MZ 16F, Leica Microsystems) was used to image the sciatic nerve and surrounding tissue. Bright field and fluorescent images were taken. One dose of fluorescein isothiocyanate labeled dextran (FITC, 30mg/kg, average molecular weight 2,000,000, Sigma Aldrich product #54271) was injected into a tail vein allowing for imaging of fluorescently labeled vasculature. Upon completion of each imaging session, a subsequent injection of atipamezole hydrochloride (0.3 mg/kg, Orion Pharma) was used to reverse the effects of dexmedetomidine anesthesia.

III. Results

We present the use of an implantable device with an attached optically clear window for use in chronic investigations into the normal and pathological functioning of peripheral nerves. Chronic window preparations are not new to the fields of neuroscience and neural engineering, although applying them to the PNS, in combination with chronic imaging techniques such as fluorescent microscopy [11], multiphoton [12], photoacoustic microscopy [13], and optical coherence tomography (OCT) [14], could allow for many novel discoveries.

The challenging aspect of chronically imaging a peripheral nerve is access and anchoring. We have developed a surgical method to address the issue of distal subcutaneous access by transposing the sciatic nerve atop resident muscles (fig. 2.). The muscle was bluntly dissected, the nerve repositioned onto muscle, and the muscle sutured so that the nerve (for example sciatic) is visible and accessible. Once the nerve was repositioned and device implanted, one could chronically image *in vivo* on a daily basis without causing damage to the nerve.

One of the challenges of moving from the central nervous system to imaging in the peripheral is the lack of bone anchoring that can be done in a limb. Our group has solved this problem in the design of our PNS window device by allowing the device to be sutured underneath the skin.

Preliminary data demonstrates device and imaging stability at one week. Further studies designed to circumvent the potential stumbling blocks of scar tissue, adhesions and motion artifact are warranted to demonstrate long-term stability of the peripheral nerve (Fig. 3.). The rat implanted with the PNW did not develop lameness or infection postoperatively, and did not traumatize the PNW or the rest of the surgical site

IV. Discussion

Chronic imaging has numerous advantages within *in vivo* imaging studies for understanding dynamic biological processes such as normal and abnormal nervous system functions. Using chronic imaging, one is able to perturb a site once without additional trauma caused by subsequent surgeries. With the usage of the PNW, various nerves of the peripheral nervous system can be accessed for observational studies. Additionally, the PNW device allows for nerve tissue, which is physiologically underneath muscular tissue, to be brought into visible range of the window via

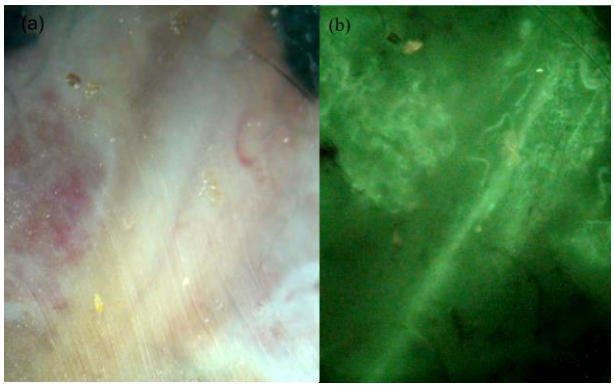

Fig. 3. Above are two in vivo images of the sciatic nerve of a rat taken on day three after implantation. (a) was taken with brightfield microscopy (b) was taken with the same microscope with a fluorescent lamp turned on after tail vein injection of FITC, a vasculature label. The nerve has one large single vessel that runs parallel with it, as well as vasculature seen within the nerve and on its periphery.

mechanisms on the device and from suturing techniques as described in the methodology section.

The PNW not only offers opportunity for chronic high resolution fluorescence imaging with brightfield and laser scanning microscopy but also can be used for new emerging techniques such as fluorescence lifetime microscopy (FLIM) to image the metabolic microenvironment [12], second harmonic generation (SHG) to image collagen deposition and remodeling [15], or optical coherence tomography [13], to obtain higher spatial views of the peripheral nerve. As well the PNW can serve as a platform for new technologies that can offer additional monitoring and intervention advantages.

Micro-electrode arrays, nerve cuffs, microfluidic channels for drug delivery, or optic fibers could all be placed beneath the window in contact with the nerve for further discovery while simultaneously being imaged on a chronic basis. Chronic imaging of the PNS while concurrently being able to record neural signals from devices placed underneath the peripheral viewing window platform would greatly enhance our knowledge of nerve anatomy and physiology as well as give us insights into PNS injury and disease. Observing the nerve in real time would allow researchers to learn more about what occurs during the complex wound healing process. Data like these might better help work being done in the fields of pain management, neuroengineering, neuroprosthetics, and neurodegenerative disorders.

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