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New Approaches to Bridge Nerve Gaps: Development of a Novel Drug-Delivering Nerve Conduit

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***Abstract*— Contemporary bridging techniques for repairing nerve gaps caused by trauma require autologous nerve grafts, which are difficult to harvest and handle and result in significant donor site deficit. Several nerve conduits with axon growth-enhancing potential have been proposed, developed and tested over the past fifteen years. In this work, prototypes of a nerve conduit designed to bridge large nerve gaps (≥10mm) end-to-end were incorporated with concentric drug reservoirs for constant and controlled drug delivery to enhance axon growth. These devices were designed, fabricated and tested *in vitro* in amber glass vials with bovine serum albumin in order to determine the drug release kinetics for future application. Our devices have shown the capability to deliver the drug of interest over a 6-day period.**

I. Introduction

Almost 3% of trauma patients suffer from peripheral nerve injuries causing lifelong disturbances [1] in function and adverse socioeconomic consequences [2] due to the fact that these injuries are difficult to treat and have poor outcomes with contemporary treatments [3]. Peripheral nerve injuries caused by accidents or battlefield incidents with nerve gaps greater than 1-2cm require special bridging techniques [4]. Autologous nerve grafts are typically used to bridge the nerve gap; however, this requires additional surgery and can result in donor site deficits in function or sensation. Despite successful tension-free re-approximation of severed nerve ends, outcomes are dismal primarily due to poor healing, scar formation and the slow rate of nerve regeneration.

Several options have emerged to improve outcomes in peripheral nerve repair. These include artificial nerve grafts, cadaver grafts and nerve conduits. Nerve conduits made with either synthetic or natural materials have been used to guide axons and bridge the nerve gaps ranging from 5 to 80mm [5]. For example, biodegradable polymer conduits embedded with Schwann cells showed a better result for nerve regeneration compared to autografts over a 6-week period *in vivo* [6]. Additionally, biodegradable polymer nerve conduits with nerve growth factor (NGF) were tested for three months *in vivo*, showing significant NGF release in a 10-mm nerve gap in a rat sciatic nerve model [7]. In brief, vascular endothelial growth factor (VEGF) [8], [9], NGF [10] and glial cell line-derived neurotrophic factor (GDNF) [11] can all stimulate nerve growth and enhance axon propagation.

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II. Design and Fabrication of the Nerve Conduit

A. Design and materials

Prototypes of the nerve conduit were designed, fabricated, and tested to explore the possibility of drug delivery using concentric conduits with a semi-permeable membrane. Polydimethylsiloxane (PDMS) was chosen to form the main structural layer of the drug reservoir, i.e., the outer concentric tube, due to its biocompatibility [12–15] and its ability to form nano-scaled structures. Ten to one PDMS to its curing agent (Sylgard 184 silicone elastomer base and curing agent, Dow Corning, Midland, MI) was used to acquire a relatively flexible PDMS structure. Pellets of thermoplastic polycarbonate-urethane (PCU), purchased from DSM Biomedical, referred to as Bionate II, were heat extruded into tubes with outer and inner diameters of 1.5 and 1.3mm, respectively. Bionate II tubes show good biocompatibility [16] and can be suitable for *in vivo* drug delivery. Biocompatible polyethersulfone membranes (PES0032005, SterliTech, Kent, WA) with 0.03μm pore size were attached to a window created in Bionate II tube in order to control drug diffusion. Figs. 1a and 1b show cross-sectional illustrations of the nerve conduit from the front and side views, respectively. These conduits would be interposed between the cut ends of a nerve. Once the drug diffuses from the reservoir via the filter, it will contact the axon growth cone and enhance axon number, diameter and density within the conduit and across the gap.

A 12-mm-long PDMS cylinder reservoir with a thinner 15-mm-long Bionate II tube was the prototype used to demonstrate diffusion across the polyethersulfone filter. The drug reservoir volume ranged between 50 to 100μL among the various prototypes. Drug release was controlled by selection of polyethersulfone membranes with different pore size or the window size on the Bionate II tube. The pore size was fixed at 0.03μm, while the window size slightly varied from one device to another due to the flexible nature of Bionate II tubes.

B. Fabrication

Ten to one PDMS was prepared using standard methods of PDMS soft lithography [17], [18]. The PDMS solution was then poured into 1.5mL Eppendorf vials and baked at 65˚C for two hours to form cylinders. A 5mm biopunch was used to create a hollow structure – the drug reservoir – in the PDMS cylinders and the same tool with a 1.5mm biopunch was used

to form the outer PDMS plugs to seal and secure the Bionate II tube in the device, as shown in Fig. 1c.

The Bionate II tubes prepared with a 1.4mm outer diameter and a 1.0mm inner diameter were used as the nerve conduit for *in vitro* drug release experiments. The tube was cut into 15mm sections and one holes with approximate 1-by-2.5-square millimeter dimension was punched at the center to serve as windows for the drug to diffuse into the tube. A polyethersulfone filter membrane was prepared in squares to cover the window and designed for the control of drug release. Loctite 4011 adhesive (18680, Loctite, Westlake, OH) was applied along the edge of the window to attach the polyethersulfone filter membrane without blocking the pores on the membrane.

The Bionate II tube with the filter membrane secured was then placed in the lumen of the PDMS cylinder. Small PDMS concentric plugs were prepared by punching a 3mm-thick PDMS layer with 5mm and 1.5mm biopunches. They were then plugged into the Bionate II tube at both ends in order to form a drug reservoir in the lumen of the PDMS cylinder. One end of the PDMS tube was sealed with this 3mm-thick PDMS plug and some uncured PDMS and baked for 1 hour at 65˚C. The drug was then injected into the reservoir through the other end of the PDMS cylinder. In order to minimize bubbles, which lead to loss of diffusion area on the filter, the drug reservoir was filled completely. Therefore, the volume of drug ranged between 50 to 100µL. Next, the other side of the PDMS cylinder was sealed with another PDMS plug, while RTV 734 silicone sealant (2307774-1008, Dow Corning, Midland, MI) was applied instead of uncured PDMS, since it required no heating, which could have damage the drug. After the device was placed at room temperature for 15 minutes to cure the sealant, it was ready to use.

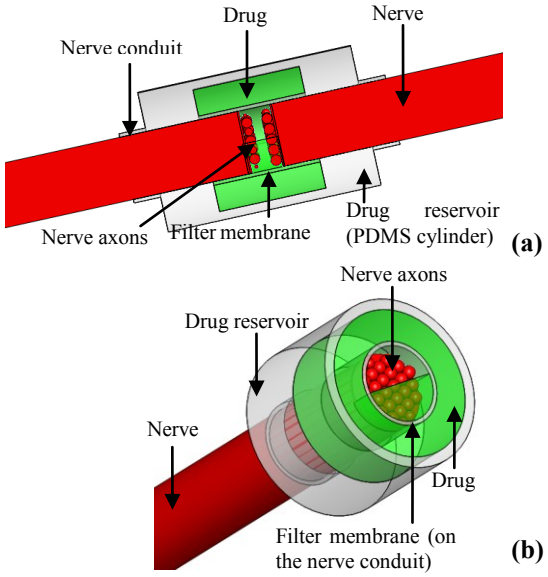
III. Test and Results

A. *Test setup*

The prototype test experiments utilized bovine serum albumin (BSA, A8022-500G, Sigma Life Science, St. Louis, MO) to demonstrate the diffusion across the device and the receiver chamber, which was a 7mL amber glass vial filled with 7mL of 1x Phosphate buffered saline (PBS). The initial concentration of the BSA solution (serving as a drug simulant) was 100mg/mL and was prepared by vortexing 100mg of BSA powder into 1mL PBS at room temperature for three minutes. Three unknown samples were tested along with three positive controls and two negative controls, as shown in Table 1. BSA standards (23209 and 23225, Thermo Scientific, Rockford, IL) were used to both prepare the positive standards between 2mg/mL to 10µg/mL and analyze the concentration on three microplates.

For negative control 1 and samples 1, 2 and 3, the 6-day diffusion test started with filling the Bionate II tube (nerve conduit) with the desired drug, as shown in Table 1, then the whole device was immersed into the 7mL of PBS in the 7mL glass amber vial. For all the positive controls, on the other hand, the desired volume of BSA was filled directly into the 7mL of PBS inside the vial. For negative control 2, since no PDMS device was used, 80µL PBS was directly injected into the 7mL PBS in the vial. Multiple positive controls were used to not only serve as controls for different samples with different volumes of BSA, but also to provide readings in extremely small concentration value to better describe the situation when the diffusion rate was limited for some devices. Positive control 1, with 120µL BSA, could serve as the control for all three unknown samples, while positive control 2, with 80µL BSA, could serve as the control for sample 2 and 3.

All vials were placed on a vial holder that sat on an orbital shaker which smoothly mixed the medium in the vials. The purpose for filling the Bionate II tube with PBS before putting the device into the vial was to eliminate air bubbles which would impede the drug from diffusing out since the polyethersulfone filter membrane was designed to deliver drugs from a solution into another and bubbles in contact with the filter membrane on the receiver chamber side would lead to no diffusion. The same reason also applied to the receiver



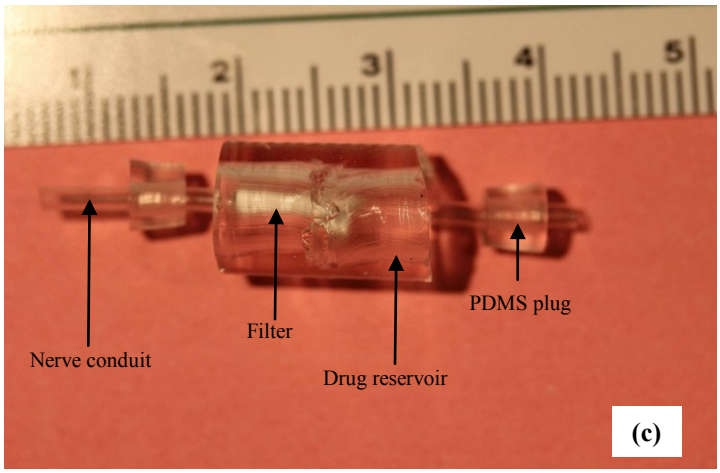


Fig. 1 Schematic diagrams of the device. (a) Schematic diagram showing membrane placement and the assembly of device on a pair of nerves. (b) Nerve axon growth in an axial view of the device. (c) Photograph of the PDMS prototype with polyethersulfone filter membrane used for BSA elution kinetics.

TABLE I. SAMPLES CORRESPONDING TO DRUG AND RECEIVER CHAMBER

Sample name	Drug in the reservoir	Medium in the receiver chamber	Maximum possible concentration in the receiver chamber
Positive control 1	120µL BSA	7mL PBS	1714.3µg/mL
Positive control 2	80µL BSA	7mL PBS	1142.9µg/mL
Positive control 3	2µL BSA	7mL PBS	28.6µg/mL
Negative control 1	80µL PBS	7mL PBS	0
Negative control 2	n/a (no reservoir)	7mL PBS	0
Sample 1	50-80µL BSA	7mL PBS	1142.9µg/mL
Sample 2	80-100µL BSA	7mL PBS	1428.6µg/mL
Sample 3	75-80µL BSA	7mL PBS	1142.9µg/mL

chamber in which the PBS level was almost full in order to minimize bubbles in the receiver chamber, and therefore minimize the possibility of bubbles appearing in the Bionate II tube.

Samples were collected after 0.5, 17, 46, 61, 91 and 112.5 hours in triplicate in three 96-well microplates. Standards, on the other hand, were prepared in duplicate in each microplate and thus six copies of one standard were acquired. Before collecting samples from vials, a 30 second vortex step was performed in order to mix the solution well. The same volume of PBS was added into the receiver chamber every time after the sample collection in order to maintain the same volume of the receiver chamber. After collecting the last series of samples, 200µL of working reagent was then added in each well and we then followed the standard operation procedure provided by the assay maker for the BSA standard to perform plate reading at 562nm wavelength on a spectrophotometer [19].

A follow up experiment was designed to determine the magnitude of the diffusion when incorporating a nerve conduit on the device. The similarity in molecular weight between 56kDa BSA [20] and human 45kDa VEGF [21] suggested that they could be used interchangeably for proof of concept, and both were much smaller than the 0.03µm pores on the filter membrane. Therefore, BSA was chosen due to its bigger size and slower diffusion, making it a worst case scenario.

B. *Results*

Readings from the spectrophotometer plate reader were analyzed and calibrated using Microsoft Excel. Fig. 2a shows the BSA concentration in the receiver chamber over the six day period, and Fig. 2b shows the percentage of BSA released into the receiver chamber in the six day period. Since the volume of BSA in the three samples were within specific ranges, the maximum possible BSA volume was used as the denominator, that is, 80µL for sample 1, 100µL for sample 2 and 80µL for sample 3.

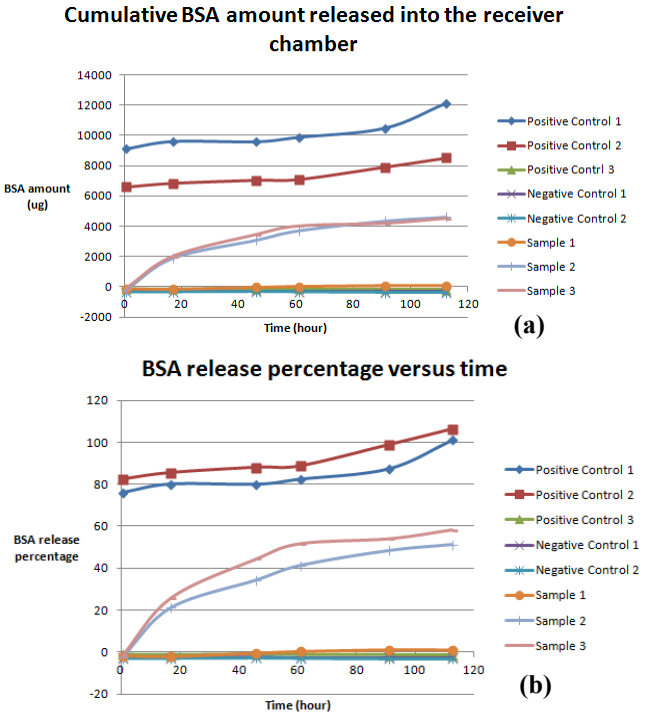


Fig. 2 Six-day BSA release into the receiver chamber. Three positive controls and two negative controls were given along with three unknown samples. (a) BSA concentration (µg/mL) measured in receiver chambers. (b) BSA release percentage compared to total amount of BSA supplied

Though the BSA release percentage of positive control 1 in Fig. 2b increased from 76 to 101% and that of positive control 2 increased from 82 to 106%, they were relatively flat compared to samples 1 and 2. The BSA release percentage of sample 2 increased 23.6% between the 0.5 and 17th hour, while the release from sample 3 increased from 27.6%. All the samples fall between positive control 1 and negative controls 1 and 2.

IV. DISCUSSION

In the BSA testing, after 61 hours of diffusion, the diffusion rates (the slope in Fig. 2b) of both sample 2 and 3 slowed down because the BSA concentration in the receiver chamber was higher and thus the diffusion gradient became lower. The diffusion rates for the first 61 hours were 0.68 and 0.85%/hr for sample 2 and sample 3, respectively. In comparison, the diffusion rates for the proceeding 51.5 hours were 0.19 and 0.13%/hr for sample 2 and sample 3, respectively.

Negative values of the BSA release percentage were generated from the negative concentration based on the calibration curve in which the standards were prepared between 0 to 2000µg/mL. Since the calibration equation was a linear function that fits most of the curve to achieve minimum standard deviation, the equation could not interpret the concentration in the region of lower concentration precisely and therefore lower concentrations than were possible were acquired for the same plate reading. If only the reading values of small concentration standards were used to generate the concentration calibration equation, the reading values of both

negative controls 1 and 2 were below the reading for 0 concentration standard, while that of samples 1, 2 and 3 were above the reading for 0 concentration standard.

Last but not least, the BSA concentration increment of sample 1's receiver chamber was about 27μg/mL during the 112.5 hours of diffusion based on the readings from lower concentration standards. Its diffusion rate was relatively low compared with samples 2 and 3 probably because the filter was blocked. Either the Loctite 4011 adhesive from the fabrication process, or air bubbles in the drug reservoir blocked the filter; therefore, the drug could not diffuse out and release into the nerve conduit, diffusing into the receiver chamber. Though the filter was blocked in sample 1, it suggests that no or minor leakage was shown on this device since the drug would not leak into the receiver chamber.

V. Conclusion and Future Work

This work suggests that the prototypes for demonstrating the possibility of drug delivery across the filter membrane and release into the nerve conduit using BSA were successful and could be used for drug delivery at a reasonable release rate. VEGF and NGF will be used to explore the capability of the device followed by *in vivo* drug delivery test on rats.

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