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Controlled Release Drug Coatings on Flexible Neural Probes

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Abstract— **We present the development, characterization and *in vivo* validation of a novel drug eluting coating that has been applied to flexible neural probes. The coating consists of drug eluting nanoparticles loaded with an anti-inflammatory drug embedded in a biodegradable polymer. The drug eluting coating is applied to flexible polymer neural probes with platinum electrodes. The drug eluting device is implanted in one hemisphere of a rat, while a control device is implanted in the opposite hemisphere. Impedance measurements are performed to determine the effect of the drug eluting coating on the tissue reaction surrounding the probe and the electrical characteristics of the devices. Probes that are coated with drug eluting coatings show better long term impedance characteristics over control probes. These coatings can be used to increase the reliability and long term success of neural prostheses.**

I. Introduction

MICROELECTRODES implanted in nervous tissue can be used to stimulate or record neural activity and hold the promise to reproduce neural functions lost to trauma or disease [1, 2]. A major problem with chronically implanted microelectrodes in the brain is that they eventually lose electrical contact with neural tissue due to the post-implantation inflammatory reaction [2-4]. Glial cells rapidly migrate to the implantation site surrounding the device thus physically separating the microelectrode sites from the neurons they are meant to be recording from or stimulating. After approximately four weeks the inflammatory response has reached its peak and bioimpedance measurements have stabilized at their maximum while the number of single unit recordings has reached its minimum [5]. If microelectrode arrays are to be effective in neural stimulation and recording, this tissue response must be reduced in order to maintain electrode-neural tissue contact.

Our approach to combat this inflammatory response involves coating microelectrode arrays with drug eluting materials. We hypothesize that highly localized and sustained drug delivery of an anti-inflammatory drug to the implantation site will reduce the response to implantation and improve recording and stimulation characteristics. In this study, novel nanoparticle embedded coatings were designed and evaluated for the controlled release of the anti-inflammatory drug dexamethasone. Dexamethasone was loaded into poly(propylene sulphide) nanoparticles which were then embedded into poly(ethylene oxide) (PEO), a biocompatible polymer, and applied as a coating to the neural probe devices. The PEO is meant to rehydrate soon after implantation thereby releasing the drug loaded nanoparticles and exposing the electrode sites. The nanoparticles protect the drug from degradation, and ensure its controlled release with over five days. The nanoparticles were examined using scanning electron microscopy for morphological changes during the coating procedure. The coating thicknesses were evaluated using scanning electron microscopy. The neural probe devices that we have developed are based on polyimide microfabrication and have been demonstrated in vivo for acute and chronic measurements [6, 7]. A novel bilateral design was developed for the reported bi-lateral measurements. Coated, drug eluting devices were implanted along with non-drug eluting devices in opposite hemispheres of adult rats. The control devices however, were coated with PEO for a proper control because PEO reduces non-specific protein interactions.

The goal of this work was to determine the efficacy of these novel drug eluting coatings to combat the inflammatory response to implantation. The efficacy was evaluated using bioimpedance measurements which measure the tissue growth electrophysiologically.

II. Materials And Methods

A. Neural Probe Fabrication

The full fabrication method has been published elsewhere but we summarize it here. Neural probes were fabricated using the method previously described by our group [6, 8]. Figure 1 briefly demonstrates the fabrication process. A polyimide layer (1.5um) is deposited on a silicon wafer. Openings are made in this layer which become metal surface electrodes. A Ti/Pt/Ti sandwich is then deposited and photolithographically defined. A final polyimide layer (40um) is deposited, defined, and etched down to the wafer thus defining the neural probe. Electrical connectors are attached and the device is released from the wafer.

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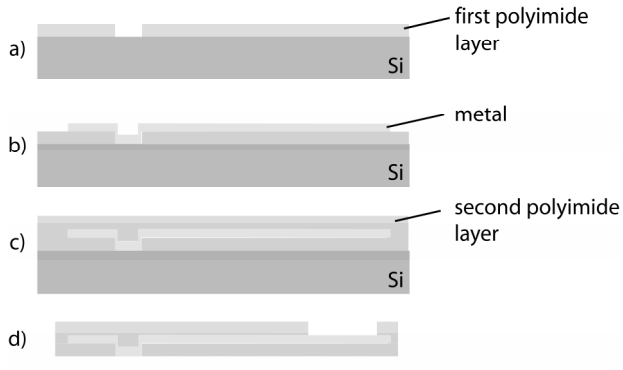

Fig. 1 – (not to scale) The neural probe microfabrication process. a) A 1.5µm polyimide layer is deposited on a silicon wafer and etched. b) The metal layer is deposited and photolithographically defined. c) A final thick polyimide layer (60µm) is deposited. c) The polyimide is etched using an oxide etch mask and d) released from the wafer.

B. Coating Preparation and Application

1) Nanoparticle Synthesis

The synthesis of the nanoparticles used has been previously described [9-11]. These nanoparticles were shown in [10] to be non-cytotoxic. All solvents and reagents were purchased from Sigma-Aldrich (Buchs, Switzerland). Deoxygenated water is flooded with nitrogen gas and 500 mg of Pluronic F-127 is added and stirred for one hour until completely dissolved. 400 uL of Propylene Sulfide is added to the solution under nitrogen purge and is allowed to mix before adding the initiator solution. The initiator consists of unprotected pentaerythritol tetrothioester and is prepared as described in [10]. Sodium methoxide in methanol is used to activate the SH end groups of the pentaerythritol tetrothioester and initiates crosslinking between the PPS molecules. 200uL of 0.5M sodium methoxide is added to the pentaerythritol tetrothioester in a nitrogen gas environment. The initiator solution is then added to the PPS and Pluronic solution and stirred for 8 hours. Upon exposure to air the PPS is crosslinked. The nanoparticle solution is then purified through a 100,000 kD dialysis membrane for several days ensuring that the solution is void of unreacted pluronic, PPS and reagents.

2) Dexamethasone Loading

The final step in nanoparticle synthesis is the drug loading. Dexamethasone was loaded using an adapted solvent-evaporation technique similar to [12]. 5 mg of dexamethasone (Sigma Aldrich, Switzerland) is added to 400 uL of methanol and stirred until dissolved. 1 mL of the nanoparticle solution described above was added and the mixture was stirred for 8 hours covered only by a particle free tissue to allow the solvent to evaporate. The solution is sterile filtered and is ready for suspension in the PEO gel.

3) Nanoparticle Imaging and Sizing

A dynamic light scattering microscope is used to measure a sample of the solution to determine the average nanoparticle size after loading. Average size was found to be 120nm in diameter. Furthermore, an environmental SEM technique (WETSEM, Quantomix Corp, Israel) is used to image the particles to determine their morphology before and after release. This ensures that the nanoparticles were not damaged during the coating procedure.

4) Nanoparticle Incorporation with PEO

1 mL of dexamethasone loaded nanoparticles are slowly added to 4 mL of a PEO (1,000,000 molecular weight) and water solution. PEO in this state is a viscous fluid. A neural probe is inserted into the mixture and quickly pulled out. The PEO-Nanoparticle suspension is allowed to air dry and the coating procedure is repeated three times. The coated neural probe can then be stored at 5°C for several days before implantation.

C. Implantation

Four adult rats were implanted bilaterally with a drug eluting coated probe in one hemisphere, and a control, PEO coated probe in the opposite hemisphere. Subjects were implanted in the primary motor cortex. Stereotaxic coordinates were set to 1.2mm anterior, ±2.5 lateral, and toothbar -3.3. Pt wire was attached to two ground screws in contact with the cortex and served as reference for the bipolar impedance measurement.

D. Impedance Measurements

The impedance of the electrode sites on the neural probes was acquired after implantation using an Agilent 4294A precision impedance analyzer connected to a PC via a National Instruments GPIB controller. Frequency sweeps were made from 100 Hz to 1MHz at 20mV and no DC offset every other day for one month.

III. RESULTS

A. Neural Probe Fabrication

The fabricated devices demonstrate excellent flexibility and robustness. An impedance of about 1MOhm at the biologically relevant frequency of 1 KHz was obtained for each electrode site. This may seem high but the electrodes are only 25um in diameter. Figure 2 demonstrates a completed device. Figure 3 demonstrates a probe tip with platinum surface electrodes before and after coating with the PEO and nanoparticle mixture.

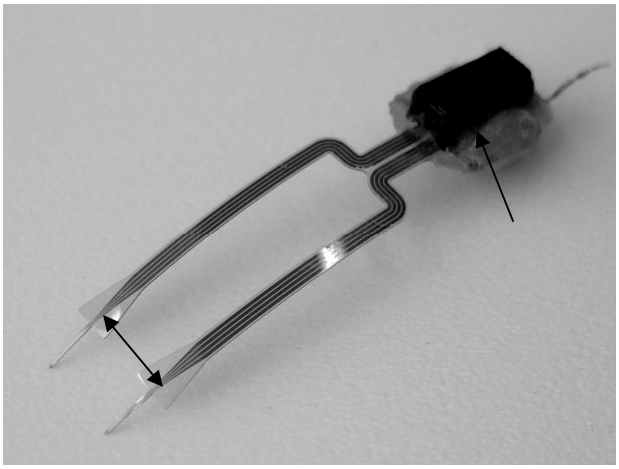

Fig. 2 The bilaterally implantable neural probe. The arrow points to the electrical connector which remains outside the animal after implantation. The double arrows span 5mm and point to the two separate probes that are implanted in each hemisphere.

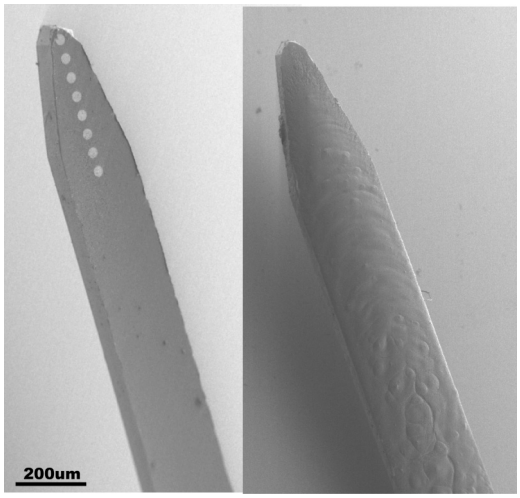

Fig. 3 - SEM image of a probe before coating (left) and after coating (right)

B. Drug Eluting Coating

The method of release and degradation of the PPS nanoparticles has been previously described. Release of dexamethasone from the nanoparticles embedded in the PEO matrix is on the order of five to seven days. This is long enough to serve in combating the inflammatory response while maintaining potency throughout several days of release. Further data will be shown on release rates from the nanoparticles before embedding in the PEO matrix and after embedding. Figures 4 and 5 compare the nanoparticles before the coating procedure, and after release from the PEO matrix. The nanoparticles have not lost their shape and have not been damaged by the coating procedure, but have grown in size. This growth is possibly due to PEO chains on the surface of the nanoparticles.

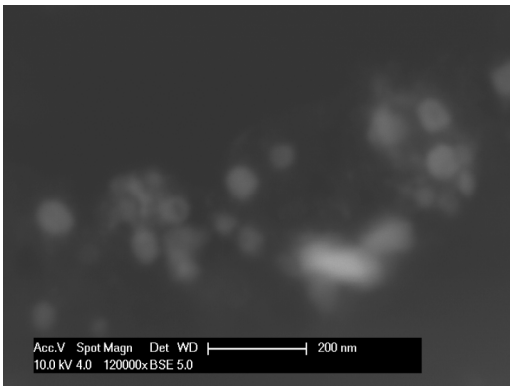

Fig. 4 – Loaded Nanoparticles suspended in saline. Average diameter is 120nm.

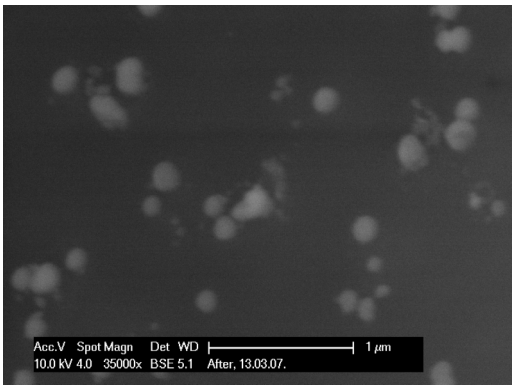

Fig. 5 – Nanoparticles after release from PEO matrix. Morphology is maintained however the nanoparticles have grown in average size.

C. Impedance Changes

The coating increases the impedance of the electrode but as it rehydrates, the electrode impedance returns to normal. Figure 6 shows an in vitro impedance measurement demonstrating a decrease over several minutes after insertion into an agar model. A small +3° increase is observed for the phase of the electrode sites but this does not affect recording/stimulation characteristics.

In vivo impedance measurements were performed to determine the effect of the coatings on the electrical properties of the electrodes. Figures 7 and 8 show the impedance magnitude at the biologically relevant frequency of 1KHz after 7 and 34 days respectively for two different animal subjects. Each graph shows the impedance of all electrodes sites, four are the drug eluting probe (right hemisphere of the brain), and four are control (left hemisphere). The trend demonstrates that drug eluting devices have lower initial impedance, and on average maintain lower impedance with time than the control devices.

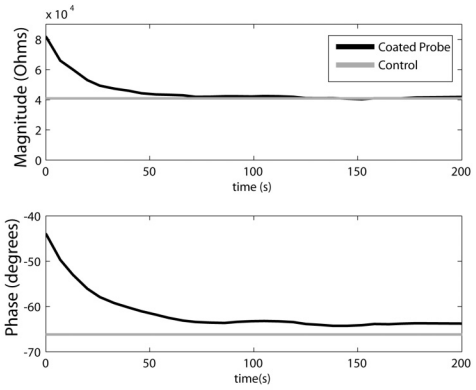

Fig 6 – An in vitro model of the impedance change after insertion in an agar model. As the PEO coating rehydrates the impedance returns to normal after only a few minutes.

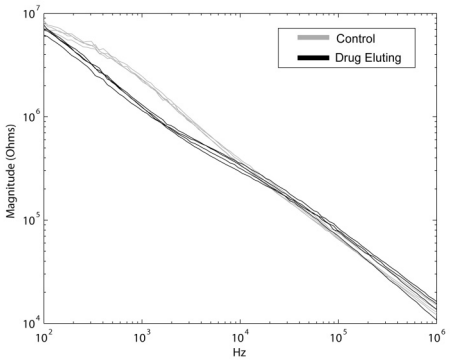

Fig. 7 - Impedance magnitude taken after 7 days. The impedance for drug eluting sites is lower than the control sites.

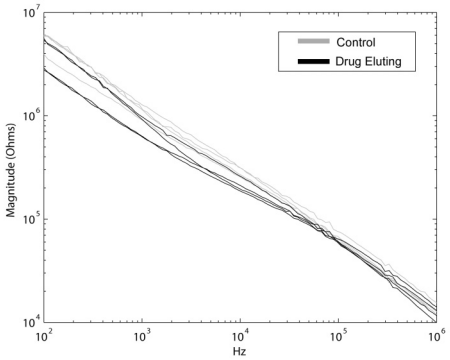

Fig. 8 - Impedance magnitude taken after 34 days (different animal subject than Figure 7). The impedance for drug eluting sites is on average lower than the control sites.

IV. Conclusion

The impedance measurements performed on the microelectrode devices demonstrate the effect the coatings have on the microelectrode's properties at the biologically relevant frequency of 1 kHz. The nanoparticles are large enough that they cannot diffuse away from the vicinity of the implanted device and demonstrate sustained release at the implantation site. The coating is applied to microfabricated polymer neural probes that would normally quickly lose their recording ability due to the tissue reaction to implantation. Drug eluting neural probes demonstrate better electrical characteristics with time and may one day offer a solution to maintaining the activity of long term neuroprosthetics.

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