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A Novel Dexamethasone-releasing, Anti-inflammatory Coating for Neural Implants

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Abstract-**The long-term stability of implanted micromachined neural probes is compromised due to the glial scar formation at the insertion site. In this study, we developed a novel nitrocellulose-based coating for the sustained local delivery of the anti-inflammatory drug dexamethasone, a synthetic glucocorticoid that effectively reduces inflammation in the CNS. *In vitro* dexamethasone release was observed over 16 days, with a relatively high release in the first three days and a slow, stable release thereafter. When Michigan neural recording probes coated with and without dexamethasone-loaded nitrocellulose coatings were implanted into the adult rat brains, immunohistochemical evidence shows a marked reduction of reactive astrocytes (GFAP), reactive microglia (ED1), and chondroitin sulfate proteoglycans (CS56) expression around the insertion site compared to uncoated probes. Impedance spectroscopy showed that the dexamethasone-loaded nitrocellulose coatings slightly reduce the magnitude of electrode impedance at the biologically relevant frequency of 1 kHz through an increase of capacitance. In vivo acute recordings demonstrate that extracellular recordings with coated probes are akin to non-coated probes and it is anticipated that with time, the coated probes will exhibit superior performance.**

In conclusion, we developed a novel nitrocellulose-based, drug releasing coating for neural electrodes that can effectively reduce scar tissue formation without adversely affecting the electrical performance of the electrodes.

I. Introduction

Stable single-unit recordings from the nervous system using Si-microelectrode arrays can have significant implications for the treatment of sensory and movement disorders. However, when these devices are implanted into brain tissue for long-term recording, they quickly (a few days to weeks) lose the ability to record neural activities due to astroglial scar formation, which is inhibitory to the neurons and forms a barrier between the electrode and neurons in the surrounding brain tissue.

To maintain long-term recording stability, reactive gliosis and other inflammatory processes around the electrode need to be minimized. The fundamental solution to this problem lies in better integration between the implanted electrode and the host nervous tissue. We propose to design a novel coating for recording probes that consists of **A) a slow release component** that is a source of anti-inflammatory agents to reduce tissue reaction; and B) **a neuro-adhesive PEI-laminin component** that allows for neuronal attachment and growth. Dexamethasone is a synthetic glucocorticoid that effectively reduces inflammation in the CNS [1,2]. In this study, we report the fabrication of a novel nitrocellulose-based coating for implanted neural probes that are capable of sustained release of dexamethasone. We further modified the nitrocellulose coatings with PEI and laminin to promote neuronal cell attachment.

II. Materials and Methods

A. Coating fabrication

100 µg dexamethasone (Sigma) powder was mixed thoroughly with nitrocellulose in methanol and evaporated on 1 cm^2 oxidized Si wafers, followed by 3 layers of evaporation of pure nitrocellulose.

B. SEM analysis

The surface morphology and thickness of the nitrocellulose coatings were investigated by scanning electron microscopy (SEM). Si wafers were coated with dexamethasone loaded nitrocellulose coatings and mounted onto metal stubs using double sided adhesive tape, vacuum-coated with a gold film, and analyzed under a LEO 1530 thermally-assisted FEG scanning electron microscope.

C. In vitro dexamethasone release assay

Si wafers coated with dexamethasone and nitrocellulose were incubated at 37°C in 1, 0.5, and 0.25 ml PBS for quantification of dexamethasone release. Every 24 h, the PBS was removed and replaced with fresh PBS. The amount of dexamethasone released during each 24 h period was determined by the UV adsorption at 242 nm with a microplate reader (Bio-Tek instruments, VT).

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D. Bioactivity of released dexamethasone

The ability of released dexamethasone to inhibit nitric oxide (NO) production by primary microglia culture activated by lipopolysaccharide (LPS) was used to evaluate the bioactivity of released dexamethasone. The cells were seeded in 96-well culture plates at a density of 3×10^4 cells per well. Twenty-four hours after seeding, LPS (1 ng/ml) and Dexamethasone released from day 1 to day 5 was added to the cell culture medium at a final concentration of 0.5 µg/ml. After 48 h, NO production was determined by measuring the accumulated levels of nitrite in the cell culture medium with Griess reagent (Promega). Cells without LPS treatment served as negative control, cells treated with LPS and 0.5 µg/ml dexamethasone served as positive control.

E. Coating modification and biocompatibility characterization by primary cortical neuron culture

0.05% (w/v) polyethylene imine (PEI) solution in borate buffer (Clonetics CC-4195 in CC-4196) was applied to the nitrocellulose coatings for 1 h, followed by 4 times of rinse with sterile water, and then the coatings were incubated with 20 µg/ml of laminin in PBS for 1 h. All the procedures were carried out at room temperature [3].

Primary embryonic cortical neurons (E17 rat) were plated on the PEI and laminin modified nitrocellulose substrates at a density of 100,000 cells/cm^2. After 8 days of culture, the neurons were fixed and stained calcein AM.

F. In vivo anti-inflammatory study

Michigan single-shank neural recording probes were coated with nitrocellulose incorporated with dexamethasone. Both coated and uncoated probes were implanted into the same brain of adult male Sprague-Dawley rats, with a coated probe in one hemisphere and an uncoated probe in the other hemisphere. After 1 week of surgery, the rats were perfused, the probes were carefully pulled out, and the brains were sectioned and immunostained with GFAP, ED1 and CS56 antibodies to identify astrocytes, reactive microglia and macrophages, and chondroitin sulfate proteoglycans (CSPGs) respectively.

The staining intensity was quantified using a matlab-based image analysis program that generated a radial array of 120 lines around the insertion site, the fluorescent intensity of all the lines was averaged as a function of distance. The integral of average intensity against distance was used to quantify the staining intensity (Fig. 1; averaged from 3 rats).

G. Impedance measurement

Micromachined silicon probes (single shank, 16 recording sites) were provided by the University of Michigan Center for Neural Communication Technology. The impedance magnitude of the recording sites was measured before and after coating. A custom built impedance measurement device was used for this study. A Tektronix TDS 3014B oscilloscope and a HP function generator were also included. The system was operated under computer control using a MATLAB program. A solution of Hank's balanced saline solution (HBSS) was used as the electrolyte. An AC sinusoid with 5 mV of amplitude was used as the input signal with the DC potential set to 0 V. The value of the impedance was determined at the biologically relevant frequency 1 kHz.

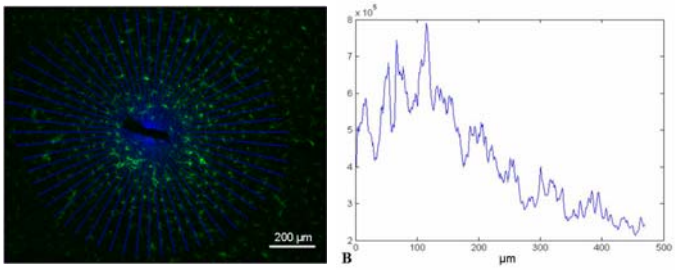

Fig. 1. The figure in (A) represents a sample image immunostained for GFAP. The corresponding radial lines measure the fluorescent intensity along the line. (B) represents the average fluorescent intensity of all the lines in figure A, with the X-axis representing the distance from the insertion site, while the Y-axis is the intensity. The area under the curve is the average intensity.

H. Acute neural recording

Acute extracellular recording of spike signals were made using standard stereotaxic recording techniques and a multichannel neural recording system (Plexon, Inc.). Briefly, rat was anesthetized and head fixed in a stereotaxic frame. Skull was exposed and a stainless steel screw was inserted to be used as a reference electrode. A 3 mm craniotomy was performed (3 mm posterior to Bregma, 3 mm lateral). Following removal of the dura, a 16-channel Michigan acute recording electrode was lowered into the brain using a stereotaxic arm with 100 micron resolution. Signals were saved in ~30 second increments when single units could be discriminated above the noise.

III. Results and Discussion

A. Physical characterization by SEM

The surface morphology of dexamethasone loaded nitrocellulose coatings before and after drug release, and plain oxidized Si wafer were investigated by SEM. As shown in Fig. 2, the coatings have porous structure, and the pores were enlarged after drug release. The thickness of the matrix coating after release is less than 0.5 µm as determined by SEM analysis (data not shown).

B. In vitro dexamethasone release

Due to its low solubility, Dexamethasone release from nitrocellulose coatings is dependent on the volume of release medium. Dexamethasone is partly water-soluble, the solubility in PBS is about 140 µg/ml. When the concentration

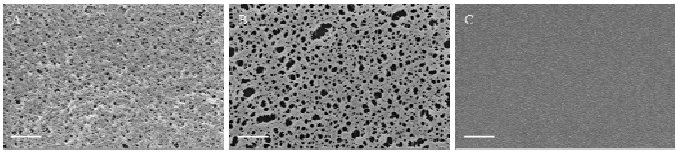

Fig. 2. Surface morphology of (A) 100 µg dexamethasone-nitrocellulose mixture coated with nitrocellulose before drug release and (B) after drug release; (C) oxidized Si surface. Scale bar = 1 µm.

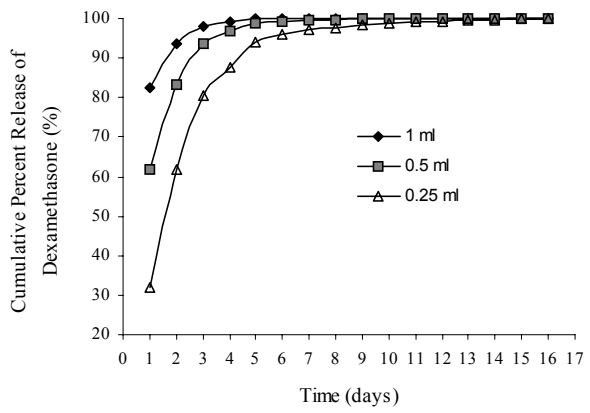

Fig. 3. Effect of the volume of release medium on release profile. Data shown are the average ± S.E.M. (n=3).

of dexamethasone in nitrocellulose coatings was higher than the saturation solubility concentration, dexamethasone release stopped after the release medium was saturated. When the concentration of dexamethasone in the coatings was lower than the saturation solubility concentration, dexamethasone release was dependent on its concentration in the release medium. As shown in Fig. 3, when volume of release medium is 1 ml, dexamethasone release only lasted for 5 days, while the release lasted for 10 days in 0.5 ml release medium. Dexamethasone release lasted for over 16 days in 0.25 ml release medium, with a relatively high release in the first three days and a slow, stable release thereafter.

C. Bioactivity of released dexamethasone

Primary microglia culture was activated by LPS (1 µg/ml), dexamethasone released on day 1,2,3,4 and 5 were added to the culture medium at a final concentration of 0.5 µg/ml. As shown in Fig. 4, the released dexamethasone is still biologically active, and the level of NO production was inhibited back to normal level when incubated with dexamethasone released every 24 h. There was no significant difference between the activity of released dexamethasone and fresh dexamethasone.

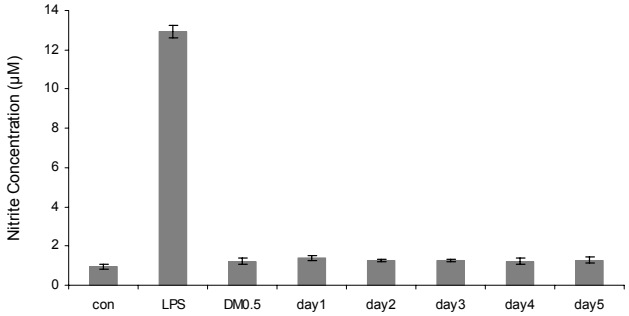

Fig. 4. Effect of dexamethasone released from nitrocellulose coatings on LPS-induced NO production. Microglia were treated with LPS, LPS and 0.5 µg/ml dexamethasone, or LPS and dexamethasone released on day1, 2, 3, 4, and 5 for 48 h, cells without LPS treatment served as negative control. *$P <$ 0.05 compared with LPS-treated cultures.

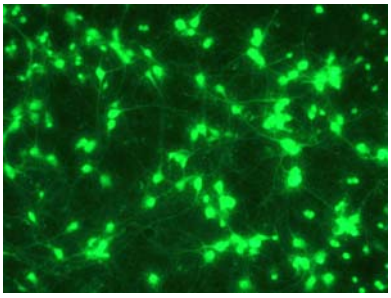

Fig. 5. Cortical neurons stained with calcein AM after 8 days of culture on Si wafers coated with PEI and laminin modified nitrocellulose. Scale bar = 100 µm.

D. Cyto-compatibility of modified coatings

The surface of nitrocellulose was modified by physical adsorption of polyethyleneimide (PEI) and laminin to improve its ability to support neuron adhesion and growth. Primary rat cortical neurons were cultured on the modified nitrocellulose coatings for 8 days and stained with calcein AM. Neurons attached to the surface and had long neurites creating a dense neuronal network. Thus, such coatings enhance neuronal integration with the electrode (Fig. 5).

E. In vivo tissue reaction

Reactive astrocytes are identified by the increased immunostaining for GFAP. 1 week after implantation, the fluorescent intensity of GFAP staining was greatly increased

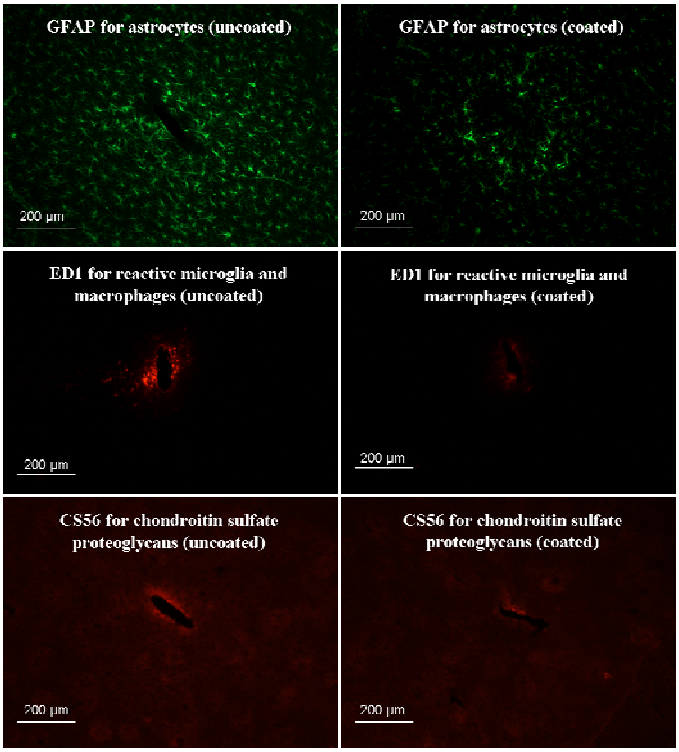

Fig. 6. nitrocellulose-dexamethasone coated electrode and uncoated electrode were implanted in the rat brain for 1 week. The left hemisphere was implanted with coated electrode, and the right hemisphere is implanted with uncoated electrode. The staining for GFAP, ED1-positive microglia and macrophages, and CSPGs was reduced in dexamethasone treated area.

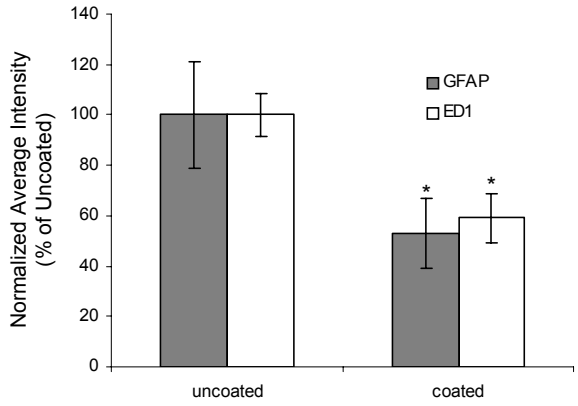

Fig. 7. The average fluorescent intensity for GFAP and ED1 staining for the uncoated probes was significantly higher than the coated probes. The average intensity was normalized relative to uncoated probes. *$P < 0.05$ compared with uncoated probes.

for the uncoated probes compared with uninjured area. However, for the dexamethasone-nitrocellulose coated probes, the number and fluorescent intensity of the GFAP-positive cells are much less compared with uncoated probes, as shown in Figs. 6 and 7.

Activated macrophages and microglia were identified by ED1 staining. ED1 expression is more restricted to the immediate area around the insertion sites compared with the GFAP staining (Fig. 5 and 6). GFAP and ED1 double staining showed that there were little GFAP positive cells in the ED1 positive area. The number of ED1 positive cells is also significantly reduced for the coated probes.

Chondroitin sulfate proteoglycans (CSPGs) are important inhibitory molecules in glial scar. The mouse IgM monoclonal antibody CS56 was used to label the chondroitin sulfate sugar epitopes [4, 5]. As shown in Fig. 6, there was an upregulation of CSPGs expression around the insertion site, and the CSPGs expression was reduced when treated with dexamethasone.

F. Impedance measurement

To determine the effects of the coatings on the electrical properties of the electrodes, the electrical impedance of the 16 recording sites on the Michigan single shank acute probes was measured with and without the coating. As shown in Fig. 8,

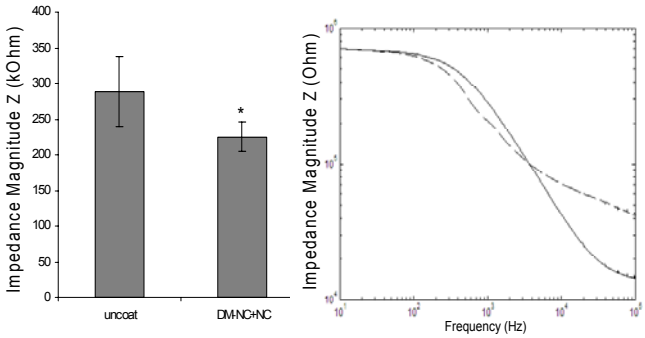

Fig. 8. Impedance magnitude of electrodes coated with dexamethasone-nitrocellulose followed by 3 layers of nitrocellulose at 1 kHz was significantly reduced compared with uncoated electrodes. Impedance spectroscopy proved the impedance drop at 1 kHz. *$P < 0.05$ compared with uncoated probes.

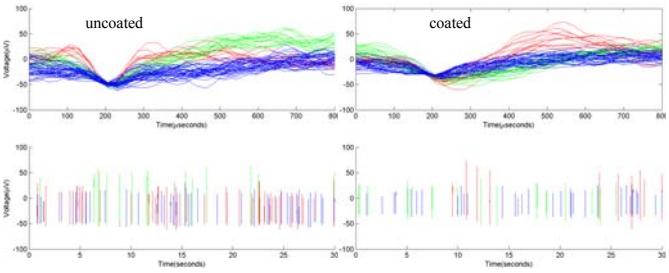

Fig. 9. Acute neural recording from rat brain for both uncoated and coated probes.

our impedance spectroscopy studies demonstrate that the magnitude of impedance at 1 kHz frequency at the recording sites was significantly reduced after coated with dexamethasone-nitrocellulose.

G. In vivo acute recording

Three single-units were sorted out from the acute neural recordings for both coated and uncoated probes (represented by red, green and blue) respectively (Fig. 9). The upper panels show the overlapping of all neural spikes generated by the three units, the bottom panels show the spikes over a 30 s time window. The magnitude of the signals recorded by the coated probes is similar to the uncoated probes.

IV. Conclusions

In this study, we report the development of a novel nitrocellulose-based drug delivery coating for neural implants. Anti-inflammatory agent dexamethasone was incorporated in this coating and slow, sustained release over 16 days was achieved. The released dexamethasone was still bioactive. This coating can effectively reduce glial scar formation *in vivo*, and the electrical performance of the electrodes was not affected by this coating. This coating can be further modified to support neuron adhesion and growth.

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