



# Controlled release of anti-inflammatory agent $\alpha$ -MSH from neural implants

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## Abstract

Si-multi-electrode arrays implanted into brain tissue for long-term recording lose electrical connectivity due to the post-implantation inflammatory reaction. This inflammatory reaction creates a physical and electrical gap between the electrode and the surrounding neurons. In this study, novel nitrocellulose-based coatings were developed for the sustained delivery of the anti-inflammatory neuropeptide  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH).  $\alpha$ -MSH was incorporated in micron-scale nitrocellulose coatings and slow, sustained release over 21 days was attained *in vitro*. The  $\alpha$ -MSH released on day 21 was still bioactive, and successfully inhibited nitric oxide (NO) production by LPS-stimulated microglia. The amount of initial drug loading directly affected the release rate, with higher initial loading increasing the mass released but not the percent of drug released. The surface morphology and thickness of the coatings were examined by scanning electron microscopy (SEM) and profilometry. In addition, impedance measurement showed that the  $\alpha$ -MSH loaded nitrocellulose coatings reduced the magnitude of electrode impedance at the biologically relevant frequency of 1 kHz. In conclusion, nitrocellulose-based, bioactive coatings that release anti-inflammatory agents without increasing the impedance of the electrode were successfully fabricated. These coatings have the potential to reduce inflammation at the electrode–brain interface *in vivo*, and facilitate long-term recordings from Si-multi-electrode arrays.

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## 1. Introduction

Stable single-unit recordings from the nervous system using Si-microelectrode arrays can have sig-

nificant implications for the treatment of a wide variety of sensory and movement disorders. However, when these devices are implanted into neural tissue for long-term recording, they quickly (a few days to weeks) lose the ability to record from neurons. Histological evidence shows that a cellular sheath surrounds the insertion site of Si-microelectrodes, which is a typical consequence of inflamma-

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tory reaction resulting from physical injury to the CNS. In the CNS this process of sheath formation is termed 'reactive gliosis' or astroglial scarring. This astroglial scar is inhibitory to neurons and forms a barrier between the electrode and neurons in the surrounding brain tissue. This problem is particularly apparent for the Michigan probes, which have relatively small recording sites and hence bigger impedance compared to the so-called Utah arrays and microwires [1–3].

To maintain long-term recording stability, reactive gliosis and other inflammatory processes around the electrode need to be minimized. The neuropeptide  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) exerts powerful anti-inflammatory effects through inhibition of proinflammatory cytokine production and related mediators of inflammation [4]. A number of studies suggest that  $\alpha$ -MSH inhibits the production of proinflammatory cytokines via the modulation of nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) activation, and the inhibition of NF- $\kappa$ B is a focal point in the mediation of the effects of melanocortins on cells of the immune system.  $\alpha$ -MSH is an excellent candidate as a powerful anti-inflammatory agent in the CNS, due to its pleiotropic effects on inflammation and energy homeostasis[5]. In this study, the fabrication of nitrocellulose-based coatings on oxidized Si substrates for the sustained release of  $\alpha$ -MSH is reported.

Conventional drug delivery systems such as microspheres are not ideal, as the limited surface area (about 1 mm<sup>2</sup>) of the microelectrodes does not allow for sufficient loading of drug carriers to facilitate sustained release. Nitrocellulose is a biocompatible polymer which has high binding capacity for proteins and nucleic acids. Although the mechanism of binding is not yet completely understood, it appears to depend mainly on hydrophobic and electrostatic interactions that are caused by the nitrate dipole [6–9]. Since nitrocellulose is a convenient substrate for rapid non-covalent attachment of proteins [10], it is commonly used to attach extracellular matrix proteins to investigate cell adhesion and growth in vitro [10–12]. NGF or laminin treated nitrocellulose has also been used in vivo to induce extensive axon growth after spinal cord injury in both adult and neonatal rats [13–15]. Although the high protein binding capacity of nitrocellulose has

enabled its extensive use both in vitro and in vivo for cell adhesion, growth and tissue regeneration, its potential for drug release has yet to be explored. In this study,  $\alpha$ -MSH was incorporated into nitrocellulose coatings and sustained release of  $\alpha$ -MSH over three weeks in vitro was achieved.

Polymer-based controlled release systems are normally classified as either reservoir (membrane) delivery systems or matrix (monolithic) delivery systems. In the former type release is controlled by a polymeric membrane that surrounds a drug containing reservoir, whereas in matrix devices the drug is either dissolved in or dispersed homogeneously throughout a polymer matrix [16–18]. Both delivery methods were investigated in this study using nitrocellulose as the polymer. The anti-inflammatory activity of  $\alpha$ -MSH released from this nitrocellulose-based delivery system was tested through nitrite production by primary microglial cells, and impedance measurement was used to characterize the contribution of the coatings to electrode impedance.

## 2. Materials and methods

### 2.1. Fabrication of $\alpha$ -MSH loaded nitrocellulose coatings

Polished Si wafers of 1 cm<sup>2</sup> with a 10,000 Å oxide layer (University Wafer) were cleaned by ultrasonification in deionized water and ethanol, and stored in 70% ethanol for sterilization. The wafers were dried under nitrogen and adhered to the spindle of a microcentrifuge (IEC) by a double sided adhesive tape. 33.3 mg (5 cm<sup>2</sup>) nitrocellulose (Schleicher and Schuell BioScience) was dissolved in 12 ml methanol; the solution (20  $\mu$ l) was then added to the surface of a wafer, followed by spinning at 2000 rpm for 30 s to enable spin coating.

#### 2.1.1. Matrix delivery method

100 or 400  $\mu$ g  $\alpha$ -MSH (Sigma) powder was mixed thoroughly with 20  $\mu$ l nitrocellulose (NC) and evaporated on 1 cm<sup>2</sup> Si wafers. This  $\alpha$ -MSH–NC layer was subsequently coated with 6 additional layers of pure nitrocellulose generated in the following manner—3 layers of evaporation and 3 layers of spin

coating (2000 rpm, 30 s). The  $\alpha$ -MSH containing layer was termed as Matrix 100 and Matrix 400 based on the amount of  $\alpha$ -MSH added to the nitrocellulose layer.

#### 2.1.2. Reservoir delivery method

100 or 400  $\mu$ g  $\alpha$ -MSH in water solution was evaporated on 1 cm<sup>2</sup> Si wafers. This  $\alpha$ -MSH layer was subsequently coated with 6 additional layers of pure nitrocellulose as previously mentioned in the matrix delivery method, as Reservoir 100 and Reservoir 400.

### 2.2. Determination of nitrocellulose and drug loading

#### 2.2.1. Nitrocellulose loading

Si wafers were weighed before coating, and then pure nitrocellulose without  $\alpha$ -MSH was applied to the Si wafers as described previously. The weight of the Si wafers was measured again, and the mass of nitrocellulose loaded onto each Si wafer was calculated by subtracting the mass of the uncoated Si wafer from that of the coated Si wafer.

#### 2.2.2. Drug loading

Si wafers were mounted on Petri dishes, and the  $\alpha$ -MSH spun away from Si wafers during spin coating was collected and quantified. The loading on the coatings was determined by subtracting the unincorporated  $\alpha$ -MSH from the total loadings (100 or 400  $\mu$ g).

### 2.3. In vitro $\alpha$ -MSH release assay

Si wafers coated with  $\alpha$ -MSH and nitrocellulose were incubated at 37 °C either in PBS for quantification of  $\alpha$ -MSH release, or in microglial cell culture medium (DMEM-F12 media supplemented with 10% fetal bovine serum) for bioactivity analysis with microglia cultures. The release medium (PBS or microglial cell culture medium) was changed every 24 h. The amount of  $\alpha$ -MSH released every 24 h from triplicate samples was determined by the subtraction of the UV adsorption at 215 and 225 nm with a microplate reader (Bio-Tek instruments, VT). The  $\alpha$ -MSH containing microglial cell culture medium collected every 24 h was stored at –20 °C until bioactivity was performed.

### 2.4. Cultures of primary microglia

Microglial cells were obtained using a modification of the procedure by Giulian and Baker [19]. Briefly, mixed glial cells dissociated from neonatal Sprague–Dawley rat cerebral hemispheres were plated in 75 cm<sup>2</sup> poly-L-lysine coated tissue culture flasks (Fisher) at a density of one brain per flask in culture medium consisting of DMEM-F12 media (Gibco) supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin. After 7–10 days, flasks were lightly shaken to release microglial cells into the media supernatant, and these floating microglia were subsequently centrifuged into a pellet, and resuspended in DMEM-F12 medium supplemented with 10% fetal bovine serum. The cells were seeded in 96-well culture plates at a density of  $3 \times 10^4$  cells per well. Twenty-four hours after seeding, microglial cells were treated with 100 pg/ml lipopolysaccharide (LPS, Sigma) and incubated in the microglial cell culture medium containing  $\alpha$ -MSH released every 24 h for 48 h.

### 2.5. Nitric oxide production

Nitric oxide (NO) production by the microglial cultures was determined by measuring the accumulated levels of nitrite in the supernatant with Griess reagent (Promega). Briefly, after incubation with LPS and the release medium collected from  $\alpha$ -MSH containing nitrocellulose coatings every 24 h for 48 h, 50  $\mu$ l cell culture supernatant was incubated with 50  $\mu$ l sulphanilamide and 50  $\mu$ l N-1-naphthylethylenediamine dihydrochloride (NED) for 10 min each at room temperature. The optical density was measured at 540 nm using a microplate reader (Bio-Tek instruments, VT). NO production with and without  $\alpha$ -MSH containing culture medium collected from triplicate nitrocellulose coating samples was determined.

### 2.6. Surface analysis by scanning electron microscopy (SEM) and profilometry

The surface morphology of the nitrocellulose coatings were investigated by SEM. Si wafers were coated with  $\alpha$ -MSH 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

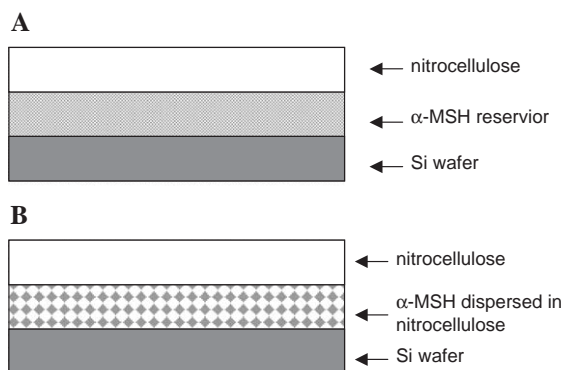


Fig. 1. Two nitrocellulose-based drug delivery methods were studied as shown in (A) reservoir delivery method and (B) matrix delivery method.

electron microscope. Coating thickness before and after drug release was measured using a DEKTAK3 profilometer (Veeco Instruments Inc.). The thickness was determined by measuring 3 random areas on each sample for triplicate samples.

### 2.7. 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 Matrix 400 coating. A custom built impedance spectroscopy device was used for this study [20]. 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.

### 2.8. Statistics

Data are represented as the average value  $\pm$  the standard error of the mean (S.E.M). Results from all experiments in this study were analyzed with the Student *t*-test, and  $P < 0.05$  was used to indicate statistical significance.

## 3. Results

### 3.1. Effect of coating methods on release kinetics

Nitrocellulose coatings containing  $\alpha$ -MSH were prepared using two delivery methods: (1) evaporated  $\alpha$ -MSH layer coated with nitrocellulose as reservoir method and (2)  $\alpha$ -MSH–nitrocellulose mixture coated with nitrocellulose as matrix method (Fig. 1). The mass of nitrocellulose loaded for reservoir coating and matrix coating was  $155.0 \pm 8.3$  and  $197.8 \pm 25.2$   $\mu\text{g}$  respectively. The actual initial  $\alpha$ -MSH loading for Reservoir 100, Reservoir 400, Matrix 100 and Matrix 400 coatings was  $97.7 \pm 6.0$ ,  $377.2 \pm 14.7$ ,  $69.2 \pm 13.5$ , and  $331.6 \pm 34.8$   $\mu\text{g}/\text{cm}^2$  respectively. Table 1 summarizes these results along with the wt.% loading of  $\alpha$ -MSH in the coatings. Active  $\alpha$ -MSH was released from nitrocellulose coatings in both delivery methods at different rates (Fig. 2). For a given initial peptide loading concentration, the matrix method had a slower release rate compared to the reservoir method. As shown in Figs. 2 and 3,  $\alpha$ -MSH was depleted at day 11 and 13 for Reservoir 100 and Reservoir 400 respectively. However,  $\alpha$ -MSH was continuously released from both Matrix 100 and Matrix 400 coatings for over 18 days.  $\alpha$ -MSH release from both types of coatings follows a similar release profile—an initial burst followed by a slow, steady release. For each type of coating, the 100  $\mu\text{g}$  initial loading demonstrated a slower release rate than 400  $\mu\text{g}$  initial loading.

Table 1  
Summary of  $\alpha$ -MSH and nitrocellulose loading

	Reservoir 100	Reservoir 400	Matrix 100	Matrix 400
Mass of drug ( $\mu\text{g}/\text{cm}^2$ )	$97.7 \pm 6.0$	$377.2 \pm 14.7$	$69.2 \pm 13.5$	$331.6 \pm 34.8$
Mass of NC ( $\mu\text{g}/\text{cm}^2$ )	$155.0 \pm 8.3$	$155.0 \pm 8.3$	$197.8 \pm 25.2$	$197.8 \pm 25.2$
Percent drug loading (wt.%) <sup>a</sup>	38.7	70.9	25.9	62.6

<sup>a</sup> Weight of drug/weight of (drug+NC).

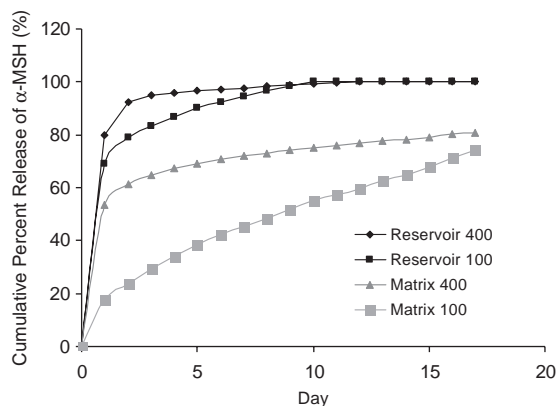


Fig. 2. Effect of coating types on the cumulative release profile. The percent  $\alpha$ -MSH loading for Reservoir 100, Reservoir 400, Matrix 100 and Matrix 400 are 38.7%, 70.9%, 25.9% and 62.6% respectively (wt.%). Data shown are the average  $\pm$  S.E.M. ( $n=3$ ).

### 3.2. Effect of initial $\alpha$ -MSH loading

#### 3.2.1. Effects of initial $\alpha$ -MSH loading on mass release

$\alpha$ -MSH released daily from nitrocellulose loaded with 100 or 400  $\mu$ g peptide was calculated as mass released (Fig. 3) and as the percent of initially loaded peptide released (Fig. 4). For the reservoir coating method, the mass released was not statistically significantly different between 100 and 400  $\mu$ g of peptide loading conditions. However, for the matrix coatings, the mass released increased with the increase in initial loading, and

the release from 100 and 400  $\mu$ g loading was significantly different ( $P<0.05$ ) after day 3. In fact, the mass release from Matrix 400 was always significantly higher ( $P<0.05$ ) than the other three groups starting from day 3. In contrast, on days 1–2 there was significantly less ( $P<0.05$ ) mass release from the matrix coatings, compared to the reservoir coatings when the initial loadings are the same, indicating slower release rate from the matrix coatings.

#### 3.2.2. Effects of initial $\alpha$ -MSH loading on percent release

For both the reservoir coatings and matrix coatings, the percent release from 400  $\mu$ g initial loading was statistically higher than release from 100  $\mu$ g on days 1 and 2. In contrast, starting from day 3, the percent release from 400  $\mu$ g was statistically lower than release from 100  $\mu$ g till day 18. These data suggest that the initial peptide loading affects the release rate; with the lower initial loading generating a slower release rate. The percent release for Matrix 100 was always significantly higher ( $P<0.05$ ) than the other three groups after day 3.

### 3.3. Bioactivity of released $\alpha$ -MSH

Primary microglial cells were treated with LPS and  $\alpha$ -MSH released into microglial culture medium from nitrocellulose coatings (Matrix 400). As shown in Fig. 5, after 21 days of release,  $\alpha$ -MSH is still bioactive,

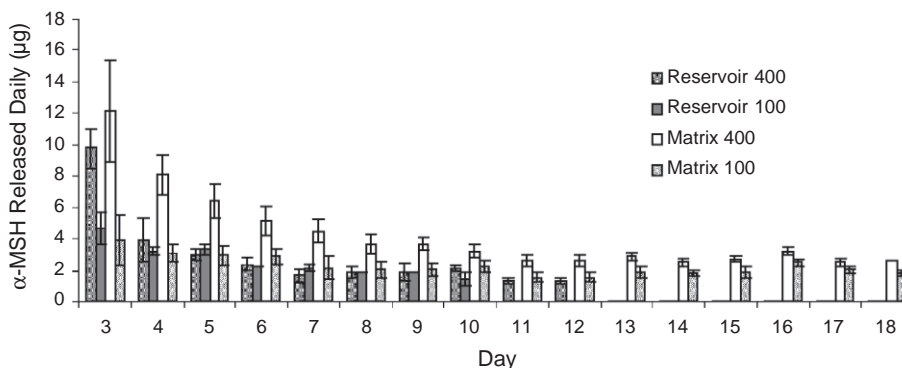


Fig. 3. Effect of initial peptide loading on mass released daily. Data shown are the average  $\pm$  S.E.M. ( $n=3$ ). The mass released from Matrix 400 was statistically significant ( $P<0.05$ ) with respect to release from the other three coatings starting from day 4 (the initial burst release on days 1 and 2 is not shown).

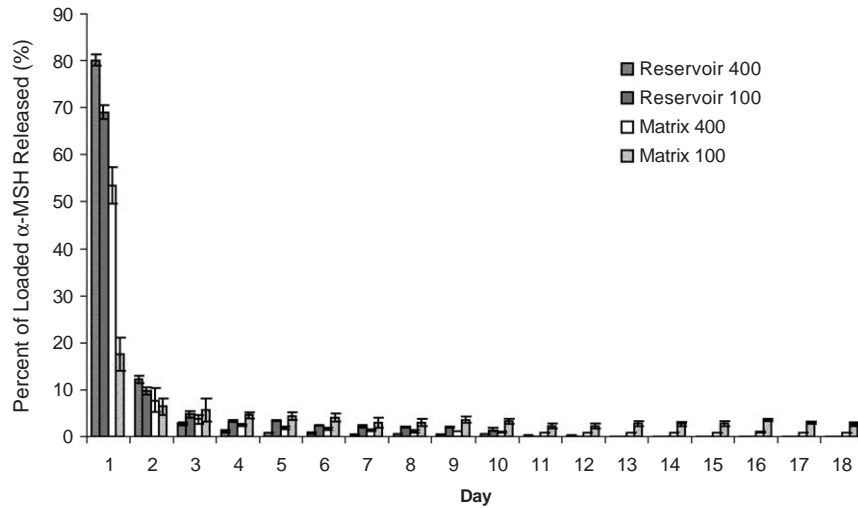


Fig. 4. Effect of initial peptide loading concentration on percent of protein released daily. Data shown are the average  $\pm$  S.E.M. ( $n=3$ ).

and the level of NO was reduced by 35% to 41% when incubated with the release medium collected every 24 h till day 21.

### 3.4. Physical characterization by SEM and profilometry

The surface morphology of  $\alpha$ -MSH–nitrocellulose (Fig. 6A, B),  $\alpha$ -MSH–nitrocellulose coated with pure nitrocellulose (Fig. 6C, D) before and after drug release, and plain Si wafer were investigated by SEM. As shown in Fig. 6, before  $\alpha$ -MSH was released, both

$\alpha$ -MSH–nitrocellulose and nitrocellulose have porous structure, with  $\alpha$ -MSH–nitrocellulose being less porous than pure nitrocellulose. After  $\alpha$ -MSH was released, the morphology of  $\alpha$ -MSH–nitrocellulose coating was very similar to pure nitrocellulose coatings, and the surface roughness was greatly reduced for both types of coatings.

The thickness of Matrix 400 coatings before and after  $\alpha$ -MSH was released was characterized. The coating thickness before drug release was  $2236 \pm 499$  nm, and the thickness after drug depletion was  $1166 \pm 143$  nm.

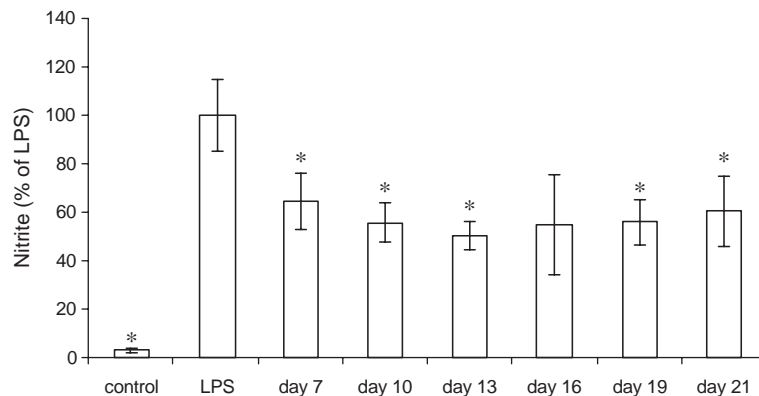


Fig. 5. Effect of  $\alpha$ -MSH released from nitrocellulose coatings on LPS-induced production of nitrite production. Data shown are the average  $\pm$  S.E.M. ( $n=3$ ). Microglia were treated with LPS, or LPS and  $\alpha$ -MSH released on day 7, 10, 13, 16, 19 and 21 for 48 h, cells without LPS treatment served as control. \* $P < 0.05$  compared with LPS-treated cultures.

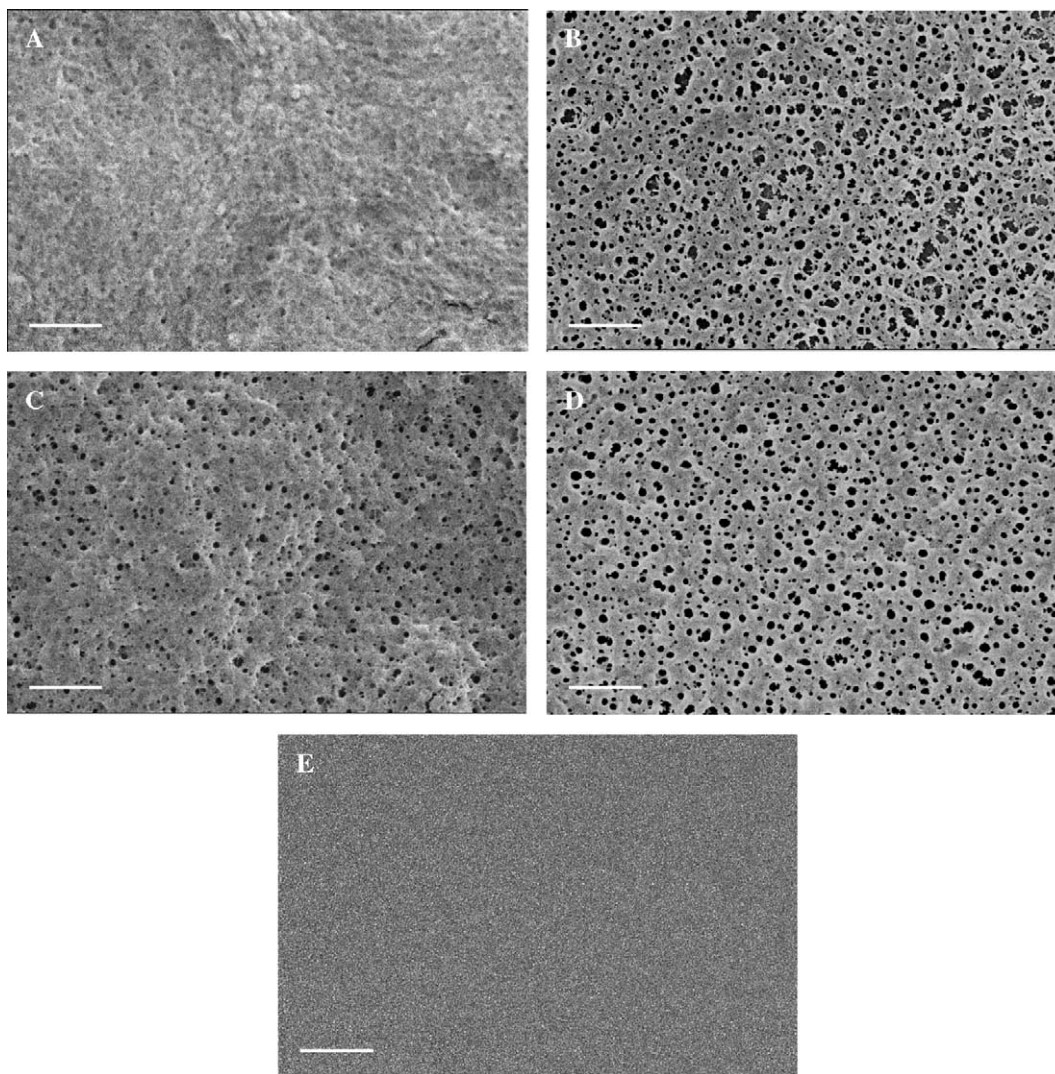


Fig. 6. Surface morphology of (A) 400  $\mu\text{g}$   $\alpha$ -MSH–nitrocellulose ( $\alpha$ -MSH–NC) before drug release, and (B) after drug release; (C) 400  $\mu\text{g}$   $\alpha$ -MSH–nitrocellulose coated with nitrocellulose ( $\alpha$ -MSH–NC+NC) before drug release and (D) after drug release; (E) Uncoated oxidized Si surface as analyzed by SEM. Scale bar=1  $\mu\text{m}$ .

### 3.5. Electronic properties

To determine the effects of the coatings on the electrical property of the electrodes, the electrical impedance of the 16 recording sites on the Michigan single shank acute probes was measured. Due to the instrument design for impedance measurement, only 14 out of the 16 recording sites could be measured. The magnitude of impedance was measured before and after coating. As shown in Fig. 7, the impedance

magnitude of the recordings sites was significantly reduced after being coated with Matrix 400 coating compared to uncoated recording sites.

## 4. Discussion

$\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) inhibits inflammation by acting on peripheral inflammatory cells, glial inflammatory cells, and on CNS

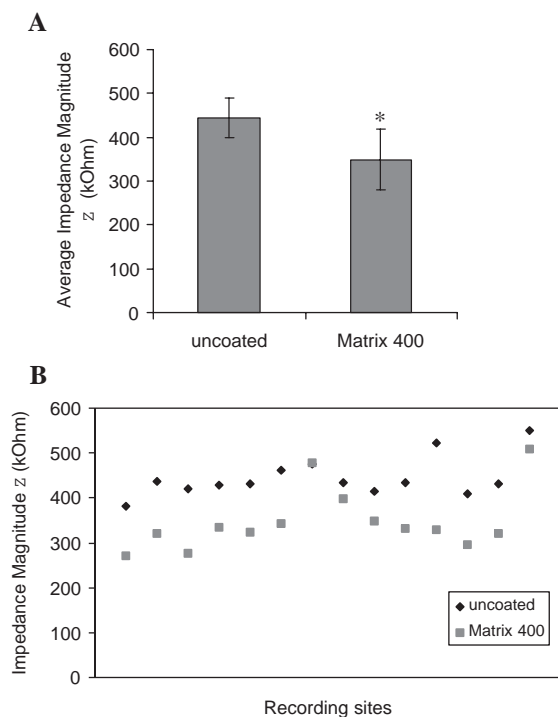


Fig. 7. (A) Average impedance magnitude for the 14 recording sites before and after coating. The magnitude of impedance for Matrix 400 was statistically significantly lower ( $*P < 0.05$ ) compared to the impedance of uncoated electrode sites. (B) Impedance magnitude for the 14 recording sites before coating (◆) and after coating (■).

receptors that activate descending anti-inflammatory neural pathways [21,22]. Therefore it is a promising agent for the treatment of the inflammatory response resulting from the implantation injury and micromotion induced scarring caused by chronically implanted electrodes. In this study,  $\alpha$ -MSH was successfully incorporated in a nitrocellulose matrix, sustained release was achieved, and the bioactivity of  $\alpha$ -MSH was retained after release.

When a matrix type loading method was used, the initial peptide loading directly affected both the mass of peptide released daily and the release rate. Higher initial loading caused a higher mass of peptide to be released every day. However, for the reservoir delivery method, higher initial loadings only caused a significantly higher mass release in the first three days, and then there was no significant difference of the mass released between initial loading amount of 400 and 100  $\mu$ g in the following days of release.

Increasing the initial loading caused a higher release rate for both coating methods.

Initial bursts of release for Reservoir 400, Reservoir 100, and Matrix 400 were observed. For the reservoir delivery method, the only barrier to slow drug release was the limiting nitrocellulose layers on top of the drug, and as shown in Fig. 6C, and the nitrocellulose layers are porous, which allow for water penetration. For the matrix delivery method, the drug was dispersed in the nitrocellulose matrix, making this layer less porous (Fig. 6A). Therefore it is not surprising that the reservoir samples have a higher initial burst compared with matrix samples when the initial loadings are the same. Also, the wt.%  $\alpha$ -MSH loading is higher for the reservoir method compared to the matrix method, and this potentially contributes to the higher initial burst observed for the reservoir samples.

For a given coating method (reservoir and matrix methods), higher initial drug loading caused higher initial release. This is most likely due to the fact that the higher drug loading increased the wt.%  $\alpha$ -MSH loading, potentially increasing the relative water permeability into the coating. In addition, higher initial loading also provided a higher concentration gradient between coatings and the release medium, which increased the driving force for drug release.

The data also demonstrate that  $\alpha$ -MSH released from the nitrocellulose-based drug delivery system remains biologically active. Any implant inserted into the brain causes tissue injury and an inflammatory response [2,23]. The initial response to CNS injury is mediated by microglia. Microglia produce inflammatory molecules such as nitric oxide, various cytokines, and prostaglandins, which induce neuronal cell death and trigger the activation of astrocytes, which finally result in the formation of an astroglial scar [24–27]. NO produced by expression of inducible nitric oxide synthase (iNOS) is an important mediator of inflammation and neuronal cell death [27,28].  $\alpha$ -MSH has been shown to inhibit pro-inflammatory cytokines and NO production [29,30]. This study demonstrates that  $\alpha$ -MSH can be successfully released for over 21 days, and remains bioactive and inhibits NO production by LPS-stimulated primary microglia (Fig. 5).

To investigate the effects of these micron-scale coatings on the electrical properties of the electrodes, impedance measurement was conducted on Michigan

single shank neural recording probes. The Michigan probes are silicon-based electrodes with 16-channel recording electrode arrays made from gold. The impedance magnitude of the electrode sites before and after being coated with Matrix 400 was measured at the biologically relevant frequency of 1 kHz. There was a significant reduction of impedance for the coated electrode sites, which consequently, improved the signal transport across the neural interface and helped to increase the detection sensitivity to neural activity [31–33]. It is interesting that nitrocellulose coatings can reduce the impedance of the gold electrodes, because nitrocellulose is a non-conductive material with the dielectric constant at 6.2–7.5. This may be related to the porous structure of the nitrocellulose coatings, which provides a high interfacial area for charge transport, helping to lower the impedance.

Several parameters of these nitrocellulose-based coatings can be altered to customize release profiles to the application of interest. The results have shown that a higher nitrocellulose–drug ratio produced a slower release profile. As the nitrocellulose coatings have a porous structure, there are two strategies to further slow drug release. First, the thickness of the nitrocellulose coatings could be increased to enhance the diffusion barrier. Second, the parameters of nitrocellulose deposition such as concentration, solvent and rate of evaporation can be changed to vary the average pore size of nitrocellulose coatings to alter the release rate.

## 5. Conclusions

In this study, the development of novel nitrocellulose-based coatings for Si-substrates/electrodes was reported. Anti-inflammatory neuropeptide  $\alpha$ -MSH was incorporated in this system and slow, sustained release over 21 days was achieved. The  $\alpha$ -MSH released on day 21 was still bioactive and successfully inhibited NO production. Compared with conventional polymer–matrix delivery systems, this novel delivery system is simple, inexpensive, and does not affect the electrical property of the substrate. In conclusion, these novel biocompatible coatings that release anti-inflammatory agents may help stabilize the electrode–brain interface to facilitate long-term recording and stimulation from Si-multi-electrode arrays in vivo.

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## References

- [1] X. Cui, J. Wiler, M. Dzaman, R.A. Altschuler, D.C. Martin, In vivo studies of polypyrrole/peptide coated neural probes, *Biomaterials* 24 (5) (2003) 777–787.
- [2] A.B. Schwartz, Cortical neural prosthetics, *Annu. Rev. Neurosci.* 27 (2004) 487–507.
- [3] J.N. Turner, W. Shain, D.H. Szarowski, M. Anderson, S. Martin, M. Isaacson, H. Craighead, Cerebral astrocyte response to micromachined silicon implantants, *Exp. Neurol.* 156 (1) (1999) 33–49.
- [4] T. Ichihama, J.M. Lipton, Autocrine  $\alpha$ -melanocyte-stimulating hormone inhibits NF- $\kappa$ B activation in human glioma, *J. Neurosci. Res.* 58 (1999) 684–689.
- [5] K. Starowicz, B. Przewlocka, The role of melanocortins and their receptors in inflammatory processes, nerve regeneration and nociception, *Life Sci.* 73 (7) (2003) 823–847.
- [6] P. Tijssen, Practice and theory of immunoassays, *Laboratory Techniques in Biochemistry and Molecular Biology*, 8th edition, Elsevier, Amsterdam, 1993.
- [7] E. Harlow, D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1988.
- [8] C. Wallis, J.L. Melnick, C.P. Gerba, Concentrations of viruses from water by membrane chromatography, *Annu. Rev. Microbiol.* 33 (1979) 413–437.
- [9] E. Handman, H.M. Jarvis, Nitrocellulose-based assays for the detection of glycolipids and other antigens: mechanism of binding to nitrocellulose, *J. Immunol. Methods* 83 (1985) 113–123.
- [10] C. Lagenaur, V. Lemmon, An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension, *Proc. Natl. Acad. Sci.* 84 (21) (1987) 7753–7757.
- [11] D.M. Snow, V. Lemmon, D.A. Carrino, A.I. Caplan, J. Silver, Sulfated proteoglycans in astroglial barriers inhibit neurite outgrowth in vitro, *Exp. Neurol.* 109 (1) (1990) 111–130.
- [12] J. Vielmetter, B. Stolze, F. Bonhoeffer, C.A. Stuermer, In vitro assay to test differential substrate affinities of growing axons and migratory cells, *Exp. Brain Res.* 81 (1990) 283–287.

- [13] D.J. Schreyer, E.G. Jones, Growth of corticospinal axons on prosthetic substrates introduced into the spinal cord of neonatal rats, *Dev. Brain Res.* 35 (1987) 291–299.
- [14] J.D. Houle, M.K. Ziegler, Bridging a complete transection lesion of adult rat spinal cord with growth factor-treated nitrocellulose implants, *J. Neural Transpl. Plast.* 5 (2) (1994) 115–124.
- [15] J.D. Houle, J.E. Johnson, Nerve growth factor (NGF)-treated nitrocellulose enhances and directs the regeneration of adult rat dorsal root axons through intraspinal neural tissue transplants, *Neurosci. Lett.* 103 (1) (1989) 17–23.
- [16] J.H. Richards, The role of polymer permeability in the control of drug release, in: J. Comyn (Ed.), *Polymer Permeability*, Elsevier Applied Science Publishers, London, 1985, pp. 217–268.
- [17] W.M. Saltzman, *Drug delivery systems, Drug Delivery Engineering Principles for Drug Therapy*, Oxford University Press, 2001, pp. 236–258.
- [18] A.Y. Polishchuk, G.E. Zaikov, Transport devices for controlled delivery, *Multicomponent Transport in Polymer Systems for Controlled Release*, Gordon and Breach Science Publishers, 1997, pp. 107–140.
- [19] D. Giulian, T.J. Baker, Characterization of amoeboid microglia isolated from developing mammalian brain, *J. Neurosci.* 6 (1986) 2163–2178.
- [20] J.D. Ross, S.M. O'Connor, R.A. Blum, E.A. Brown, S.P. DeWeerth, Multielectrode impedance tuning: reducing noise and improving stimulation efficacy, 26th Annual International Conference IEEE Engineering in Medicine and Biology Society (EMBS), September, 2004, 2004.
- [21] J.M. Lipton, H. Zhao, T. Ichiyama, G.S. Barsh, A. Catania, Mechanisms of anti-inflammatory action of  $\alpha$ -MSH peptides. In vivo and in vitro evidence, *Ann. N. Y. Acad. Sci.* 20 (1999) 173–182.
- [22] J.M. Lipton, A.P. Catania, Anti-inflammatory actions of the neuroimmunomodulator  $\alpha$ -MSH, *Immunol. Today* 18 (3) (1997) 140–145.
- [23] E. Fournier, C. Passirani, C.N. Montero-Menei, J.P. Benoit, Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility, *Biomaterials* 24 (2003) 3311–3331.
- [24] J.W. Fawcett, R.A. Asher, The glial scar and central nervous system repair, *Brain Res. Bull.* 49 (6) (1999) 377–391.
- [25] C.M. Liberto, P.J. Albrecht, L.M. Herx, V.W. Yong, S.W. Levison, Pro-regenerative properties of cytokine-activated astrocytes, *J. Neurochem.* 89 (5) (2004) 1092–1100.
- [26] J. McGraw, G.W. Hiebert, J.D. Steeves, Modulating astrogliosis after neurotrauma, *J. Neurosci. Res.* 63 (2) (2001) 109–115.
- [27] J.M. Kim, D. Son, P. Lee, K.J. Lee, H. Kim, S.Y. Kim, Ethyl acetate soluble fraction of *Cnidium officinale* MAKINO inhibits neuronal cell death by reduction of excessive nitrite oxide production in lipopolysaccharide-treated rat hippocampal slice cultures and microglia cells, *J. Pharmacol. Sci.* 92 (2003) 74–78.
- [28] S. Golde, A. Coles, J.A. Lindquist, A. Compston, Decreased iNOS synthesis mediates dexamethasone-induced protection of neurons from inflammatory injury in vitro, *Eur. J. Neurosci.* 18 (2003) 2527–2537.
- [29] R. Delgado, A. Carlin, L. Airaghi, M.T. Demitri, L. Meda, D. Galimberti, P. Baron, J.M. Lipton, A. Catania, Melanocortin peptides inhibit production of proinflammatory cytokines and nitric oxide by activated microglia, *J. Leukoc. Biol.* 63 (6) (1998) 740–745.
- [30] D. Galimberti, P. Baron, L. Meda, E. Prat, E. Scarpini, R. Delgado, A. Catania, J.M. Lipton, G. Scarlato,  $\alpha$ -MSH peptides inhibit production of nitric oxide and tumor necrosis factor- $\alpha$  by microglial cells activated with  $\beta$ -amyloid and interferon  $\gamma$ , *Biochem. Biophys. Res. Commun.* 263 (1) (1999) 251–256.
- [31] X. Cui, V.A. Lee, Y. Raphael, J.A. Wiler, J.F. Hetke, D.J. Anderson, D.C. Martin, Surface modification of neural recording electrodes with conducting polymer/biomolecule blends, *J. Biomed. Mater. Res.* 56 (2) (2001) 261–272.
- [32] X. Cui, D.C. Martin, Electrochemical deposition and characterization of poly(3,4-ethylenedioxythiophene) on neural microelectrode arrays, *Sens. Actuators, B, Chem.* 89 (2003) 92–102.
- [33] D.A. Robinson, The electrical properties of metal microelectrodes, *Proc. IEEE.* 56 (6) (1968) 1065–1071.