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Actively controlled release of Dexamethasone from neural microelectrodes in a chronic *in vivo* study

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ABSTRACT

Stable interconnection to neurons *in vivo* over long time-periods is critical for the success of future advanced neuroelectronic applications. The inevitable foreign body reaction towards implanted materials challenges the stability and an active intervention strategy would be desirable to treat inflammation locally. Here, we investigate whether controlled release of the anti-inflammatory drug Dexamethasone from flexible neural microelectrodes in the rat hippocampus has an impact on probe-tissue integration over 12 weeks of implantation. The drug was stored in a conducting polymer coating (PEDOT/Dex), selectively deposited on the electrode sites of neural probes, and released on weekly basis by applying a cyclic voltammetry signal in three electrode configuration in fully awake animals. Dex-functionalized probes provided stable recordings and impedance characteristics over the entire chronic study. Histological evaluation after 12 weeks of implantation revealed an overall low degree of inflammation around all flexible probes whereas electrodes exposed to active drug release protocols did have neurons closer to the electrode sites compared to controls. The combination of flexible probe technology with anti-inflammatory coatings accordingly offers a promising approach for enabling long-term stable neural interfaces.

1. Introduction

The development of implantable microelectrodes has revolutionized the field of biomedical applications by enabling bi-directional communication with neural tissue at high resolution. In particular penetrating probes, extending deep into neural structures, allowed substantial progress in the understanding of neuronal pathways and networks [1–4]. They further promoted therapeutic and diagnostic applications like deep brain stimulation (DBS) for treating symptoms of Parkinson's disease or severe psychiatric disorders [5,6]. In addition, brain machine interfaces (BMIs) and associated neuro-prosthetic devices rely on signals recorded from or delivered across such neural electrodes [7–9].

In chronic recordings of electrophysiological signals with penetrating probes it has however been observed that signals attenuate or disappear after only few weeks post implantation [10,11]. This effect is attributed to a foreign body reaction taking place around any object inserted into tissue. In consequence to the insertion trauma along with the disruption of the blood brain barrier as well as the presence of the probe itself, a complex cascade of immune reactions is triggered [12]. These processes can be summarized into two phases relevant for neural probes [11]: An increased concentration of activated microglial cells around the probe, identified by the ED-1 antibody, is characteristic for the acute inflammation phase immediately following the implantation [13]. With progressing time, this acute inflammation develops into a chronic state which is defined by a dense astrocytic scar tissue (identified by glial fibrillary acidic protein (GFAP)) encapsulating the probe [11,14,15]. Along with the accumulation of scar tissue over several weeks, a reduction in the number of neurons in close proximity to the implanted probe has further been observed within a radius of ca. 100 μm around the probe [14,16]. It is to date still unknown whether this loss is caused by the migration of neurons away from the implanted probe, whether neurons are repelled

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during the formation of the scar tissue or whether they eventually degenerate by other means in this process. This chronic inflammation state and the loss of close-by neurons impairs the long-term functionality of microelectrodes and ultimately leads to reduction of recorded signal quality. Under these conditions, electrical stimulation parameters need to be continuously adjusted to retain efficient tissue activation over time [10,11,17,18]. For silicon based implants, chronic inflammatory reactions have been found in tissue extending as far as 300 μm [19] or 10 mm [20] away from the probe surface beyond 16 weeks of implantation in the rat or macaque cortex, respectively. In contrast, stab-wounds did not show any persistent inflammation, demonstrating that the probe itself rather than the implantation trauma leads to the chronic inflammation state [19]. Variations in probe geometries and implantation techniques were further not found to result in improved chronic tissue response [21–23].

In order to attenuate the immune reactions towards implanted probes, flexible substrates were introduced aiming at minimizing the mechanical mismatch between the tissue and the probe [24,25]. With this approach, micro-motion associated inflammation was addressed which occurs in consequence to microscopic movements of stiff implants inside soft tissue [11]. Flexible substrate materials, such as polyimide (PI) with a Young's Modulus of ~9 GPa, were reported to provide lower tissue reactions when compared to scar formation described in the literature for stiff silicon implants (Young's modulus of ~180 GPa) [26–28]. Precise insertion of such flexible probes into the cortex however is demanding and requires complex insertion techniques, posing limitations to this approach [29–31]. Attaching biomolecules to the surface of stiff silicon implants has further been shown to reduce neuronal loss in the close vicinity to the probe [32].

Aiming at modifying the biological side rather than the probe itself, delivery of anti-inflammatory drugs has been described as strategy for attenuating chronic immune reactions and was in particular suggested to slow down neurodegeneration [19]. This technology can be used independent of the probe geometry or type and offers the possibility to directly influence the cellular reactions in the close vicinity to the implanted probe. Dexamethasone (Dex) is the most prominent corticosteroid drug described for inflammation attenuation. Several studies demonstrated reduced inflammation when Dex was released from passively eluting substrates (e.g. PLGA microspheres) implanted subcutaneously [33–36] or in the central nervous system [37–42] for times between 6 h and 28 days. The pharmacological efficacy of the drug was not influenced by the storage and subsequent release [36,38]. It has nevertheless been observed that the chronic immune response could not be affected by such passive eluting systems for implantation times beyond 30 days [39,43–45]. This observation demonstrates that pharmacological modulation of the tissue response during the acute inflammation phase is not sufficient but rather sustained delivery technologies with active control over release are required to address the chronic immune reaction.

Conducting polymers (CPs) like poly(3,4-ethylenedioxythiophene) (PEDOT) have been described as promising material for such active release technology in biomedical applications. Introduced as electrode coatings with low impedance and high charge injection capacity for neural probes [46–49], CPs further provide the possibility to incorporate, and thus store, ionic substances such as drugs in their bulk. Controlled release of stored drugs can subsequently be achieved by applying an electrical signal to the CP which enables high temporal control over drug expulsion [50–52]. Proof of concept for actively triggered release of Dex from such systems has been demonstrated *in vitro* from large substrates (0.1–4 cm^2) and for comparably short time frames (minutes to hours) [53–59]. Active drug release *in vitro* over longer time frames (up to four weeks) has been reported by us in a previous study [60] suggesting that chronic immune response modulation with such release technology is generally feasible. Chronic *in vivo* studies employing micro-scale probes with active drug delivery functionality tested over several months have however not been reported in literature so far. It is therefore still unknown whether such CP release systems provide an efficient means for attenuating the chronic immune response to implanted neural probes at time frames and in dimensions more relevant for clinical applications.

We here target this question by combining the beneficial properties of a flexible neural probe with the active drug delivery properties of CPs. The impact of this probe technology on the chronic immune response is evaluated over 12 weeks *in vivo* employing weekly triggered active release of Dex from PEDOT/Dex coated electrode sites in the rat brain. Electrophysiological, electrochemical and immunohistochemical data are collected from functionalized probes and non-functionalized controls implanted bilaterally in the hippocampus of 12 rats. Thereby techniques promoting stable long-term adhesion for CP coatings as well as strategies for enabling precise insertion of polyimide flex-probes are employed.

2. Materials and methods

2.1. Basic electrode fabrication

Neural probes for *in vitro* and *in vivo* measurements were based on flexible polyimide substrates with a platinum metallization embedded between two polyimide layers, fabricated according to the process described by Stieglitz et al. [24]. In brief, a 5 μm thin polyimide layer was deposited on a carrier wafer. For adhesion promotion, 50 nm of Silicon Carbide (SiC) were deposited on the polyimide in a chemical vapour deposition process [61]. Subsequently, 100 nm of platinum and 400 nm IrOx were sputtered on the polymer substrate and photo-lithographically patterned to define the electrode sites. IrOx thereby served as adhesion promoter for the subsequent PEDOT coatings [62]. Finally, a second polyimide layer of 5 μm thickness was deposited as insulation layer and selectively opened via reactive ion etching to create the electrode sites as well as the contact pads. The resulting flex-probes were 10 μm thin with a shaft of 380 μm width and 15 mm in length. The probes featured 16 individual electrode contacts with 12 electrode sites of 15 × 15 μm^2 and four electrode sites of 50 × 50 μm^2 according to the layout in Fig. 1a. A large ring-aperture, reinforced with a circular platinum electrode, was integrated at the tip of each electrode (Fig. 1a) to serve as guidance for an insertion tool during implantation. The distal end of the probe was designed to fit into a conventional ZIF-connector.

2.2. Deposition of PEDOT/Dex coatings

Aiming at attenuating the foreign body response, the four large electrodes in the midline of the probe shaft were selected for carrying an anti-inflammatory coating. For being able to actively modulate the drug expulsion, this coating was based on the conducting polymer PEDOT. The corticosteroid Dexamethasone 21-phosphate disodium salt (Dex) was incorporated into the PEDOT during the polymerization process and was thus stored in a confined area on the probe. The coating was realized by galvanostatic electropolymerization using an Autolab potentiostat (PGSTAT128N, Metrohm, Filderstadt, Germany) in a three electrode configuration with a stainless steel counter electrode (2 cm^2) and an Ag/AgCl reference electrode. An aqueous solution of 0.01 M EDOT and 0.01 M Dex was used as electrolyte and the deposition was carried out at a current density of 800 $\mu A/cm^2$ with a target