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Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons

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ABSTRACT

Sensorineural hearing loss is associated with gradual degeneration of spiral ganglion neurons (SGNs), compromising hearing outcomes with cochlear implant use. Combination of neurotrophin delivery to the cochlea and electrical stimulation from a cochlear implant protects SGNs, prompting research into neurotrophin-eluting polymer electrode coatings. The electrically conducting polypyrrole/*para*-toluene sulfonate containing neurotrophin-3 (Ppy/pTS/NT3) was applied to 1.7 mm^2 cochlear implant electrodes. Ppy/pTS/NT3-coated electrode arrays stored 2 ng NT3 and released 0.1 ng/day with electrical stimulation. Guinea pigs were implanted with Ppy/pTS or Ppy/pTS/NT3 electrode arrays two weeks after deafening via aminoglycosides. The electrodes of a subgroup of these guinea pigs were electrically stimulated for 8 h/day for 2 weeks. There was a loss of SGNs in the implanted cochleae of guinea pigs with Ppy/pTS-coated electrodes indicative of electrode insertion damage. However, guinea pigs implanted with electrically stimulated Ppy/pTS/NT3-coated electrodes had lower electrically-evoked auditory brainstem response thresholds and greater SGN densities in implanted cochleae compared to non-implanted cochleae and compared to animals implanted with Ppy/pTS-coated electrodes ($p < 0.05$). Ppy/pTS/NT3 did not exacerbate fibrous tissue formation and did not affect electrode impedance. Drug-eluting conducting polymer coatings on cochlear implant electrodes present a clinically viable method to promote preservation of SGNs without adversely affecting the function of the cochlear implant.

1. Introduction

Cochlear implants provide auditory perception to profoundly deaf individuals with a sensorineural hearing loss by electrically stimulating spiral ganglion neurons (SGNs) via an electrode array implanted into the scala tympani of the cochlea. Biphasic current pulses of varying amplitude, pulse width and rate are sent to the electrodes arranged according to frequency in the cochlea to provide loudness, pitch and temporal cues for speech understanding. SGNs in the vicinity of the stimulated electrode depolarize in response to the delivered charge. However, the loss of hair cells responsible for the sensorineural hearing loss leads to secondary degeneration and apoptosis of SGNs [1]. This is due in part to the loss of neurotrophic support that hair cells would normally provide. It is anticipated that extensive SGN degeneration would limit the functionality of the cochlear implant since fewer neurons would be available to transmit the information to the brain [2]. Further exacerbating the problem, cochlear implantation itself can cause the loss of residual hair cells that may otherwise have provided supplementary acoustic hearing and improved speech recognition after cochlear implantation, especially in the presence of background noise [3].

Experimentally, direct slow-rate administration of the neurotrophins brain-derived neurotrophic factor (BDNF) and/or neurotrophin-3 (NT3) into the scala tympani of the cochlea over the course of several weeks preserved SGNs after sensorineural hearing loss induced by aminoglycosides or noise [4–8]. Neuroprotective effects were limited to the period of neurotrophin delivery and were lost once treatment ceased [9]. Electrical stimulation via a cochlear implant has also been reported to maintain SGN survival after hearing loss [10–14] but other studies find that electrical stimulation alone does not provide enough trophic support for SGN survival [15,16]. However, simultaneous co-administration of

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electrical stimulation and neurotrophins provided greater SGN survival after sensorineural hearing loss than either treatment alone [16,17]. Furthermore, continuation of electrical stimulation beyond the period of neurotrophin delivery to the cochlea maintained the protective effects on SGNs [18]. Therefore, improved SGN survival after sensorineural hearing loss may be achieved via a device that provides prolonged neurotrophin treatment in addition to electrical stimulation.

Neurotrophin delivery via a cannula and electrical stimulation via a cochlear implant have previously been incorporated into a single device to examine the effect of combined treatment on SGN survival [19]. However, the device had a limited drug reservoir that would require frequent changes if drug delivery were to be continuous or surgical removal of the device if treatment were to cease. Furthermore, the drug delivery cannula may also have provided an additional route for bacteria to enter the cochlea. Safer drug delivery options that reduce the risk of infection by providing slow-release neurotrophic support from a single application need to be considered. Recent interest has focussed on drug eluting polymers, such as polypyrrole (Ppy), which may be used to coat cochlear implant electrodes. Ppy is an electroactive polymer consisting of pyrrole monomers held together by negatively charged dopants. Additional factors such as enzymes, DNA or cells can be incorporated and released from Ppy [20]. The main advantage of Ppy over other polymers is that release of incorporated molecules can be induced and controlled with electrical stimulation. For example, dexamethasone was released by cyclic voltammetry from Ppy/dexamethasone-coated neural recording electrodes with the aim of inhibiting scar formation at the electrode insertion site in the brain [21]. Likewise, electrical stimulation enhanced the release of heparin from Ppy/heparin two-fold for anti-coagulation applications in implanted devices [22].

We previously created Ppy films using *para*-toluene sulfonate (pTS) as the negatively charged dopant with or without incorporated NT3 (Ppy/pTS and Ppy/pTS/NT3). NT3 slowly diffused from the polymer in the absence of electrical stimulation, but a large increase in NT3 release occurred when a physiologically relevant electrical stimulus was applied [23]. To test the biological properties of Ppy/pTS and Ppy/pTS/NT3, auditory nerve explants were cultured from rat cochleae and grown directly on the two polymers. Explants grown on Ppy/pTS/NT3 sprouted significantly more neurites than explants grown on Ppy/pTS, demonstrating that the released NT3 was biologically active. Electrical stimulation of Ppy/pTS/NT3 boosted the release of NT3 and neurite outgrowth from explants even further [24].

This paper describes the chronic implantation of Ppy/pTS/NT3-coated electrodes into deafened guinea pig (GP) cochleae to observe effects on SGNs. This study provides the first report of a conducting polymer coating on cochlear implant electrodes being used to preserve SGNs and provide activation of central auditory pathways *in vivo*.

2. Materials and methods

2.1. In vitro NT3 release kinetics

General kinetics of ^{125}I-labelled NT3 release from Ppy/pTS/^{125}I NT3 polymers were determined as described previously [23]. Briefly, Ppy/pTS/NT3 was synthesised for 60 min on 1 cm^2 strips of gold-coated Mylar sheets using ^{125}I-labelled NT3 (ProSearch International, Australia). The polymers were rinsed several times in MilliQ water and placed in a gamma counter for initial ^{125}I NT3 incorporation readings. The polymers were then incubated in saline solution for 7 days at room temperature. Electrodes were divided into two groups; no electrical stimulation or continuous electrical stimulation (1 mA/cm^2 biphasic current pulses) using a stainless steel mesh counter electrode. The saline was collected from each well on each day and replaced with new saline. At the end of 7 days, all samples were read in a gamma counter to assay the amount of ^{125}I NT3 present.

2.2. Electrode arrays

Four-ring platinum electrode arrays specially designed for implantation in GPs were produced in-house using materials obtained from Cochlear Ltd. All electrode rings were 0.3 mm in width and had tapering diameters from 0.47 mm at the base (E4) to 0.41 mm at the tip of the electrode array (E1). Total electrode surface area for the four electrodes was 1.658 mm^2. Electrode rings were set in a silastic carrier with individual stainless steel lead wires and gold pin connectors (Fig. 1a). Electrodes were cleaned by sonication and sterilised before polymer coating and implantation.

2.3. Impedance measurements

The impedance of all electrode arrays was measured prior to Ppy-coating or implantation. The four platinum electrodes were electrically shorted and placed in a 50 ml solution of sterile phosphate buffered saline together with a return electrode that had a larger total surface area. A biphasic current pulse (3 mA current, 50 μs phase and 25 μs interphase gap) was passed between the two electrodes and the resulting voltage waveform was recorded on an isolated digital oscilloscope (see Fig. 3a). Voltage was recorded from two points: 7 μs from the beginning of the first phase and the end of the first phase (total voltage; V_t). Extrapolation of a line between these two points was used to predict the voltage at 0 μs (access voltage; V_a). Polarisation voltage (V_p) was calculated using $V_p = V_t - V_a$. These values were then used to calculate total impedance (Z_t), access impedance (Z_a), and polarisation impedance (Z_p) using Ohm's law ($Z = V/I$). After coating with Ppy/pTS or Ppy/pTS/NT3, the impedance values of each electrode array were measured again in the same way.

2.4. Synthesis of Ppy on electrode arrays

To coat the 4-ring platinum electrode arrays with Ppy/pTS or Ppy/pTS/NT3, the electrode arrays were placed in a 2-electrode set-up in which the platinum rings were electrically connected to form one electrode and a ring of stainless steel mesh was placed around the array as a common counter electrode. Under sterile conditions, the electrode array was placed in a solution of 0.2 M pyrrole, 0.05 M pTS and 2 μg/ml NT3. A constant current was applied at 2 mA/cm^2 for 40 min. After polymerization, coated electrodes were rinsed several times in MilliQ water and stored in MilliQ water at 4 °C for no longer than 4 days before use.

2.5. Guinea pigs

Male or female adult pigmented Dunkin–Hartley GPs averaging 491 ±19 g were used. National Institutes of Health (NIH) guidelines for the care and use of laboratory animals were observed (NIH Publication #85-23 Rev. 1985). The Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital approved the care and use of the animals in this study (ethics #06/131A). GPs were randomly assigned to 4 groups (Table 1).

2.6. Auditory brainstem responses

At least one week prior to deafening, an auditory brainstem response (ABR) was performed under injected anaesthesia induced by intramuscular 3:1 ketamine/xylazine mixture (60 mg/kg ketamine; Parnell Laboratories, Australia and 4 mg/kg ilium xylazil-20; Troy Laboratories, Australia). Computer generated acoustic rarefaction clicks were delivered to the anaesthetised GPs via a loudspeaker placed 10 cm from the pinna. ABRs were recorded differentially using subcutaneous needle electrodes placed at the vertex and the nape of the neck, with an additional needle electrode in the thorax as a ground. Normal hearing was defined by a threshold <43 dB peak equivalent sound pressure level (SPL). Another ABR was performed after deafening just prior to the implantation procedure to confirm deafness, defined as threshold shifts of >50 dB.

2.7. Deafening

Under gaseous anaesthetic (induced with 2% isofluorane and 1% oxygen and maintained with 1.5% isofluorane and 0.5% oxygen), the jugular vein was exposed and cannulated for the intravenous administration of the loop diuretic frusemide (100 mg/kg; Troy Laboratories, Australia). This was followed by a subcutaneous injection of the aminoglycoside kanamycin sulphate (400 mg/kg; Sigma–Aldrich, Australia). The wound was closed with tissue adhesive. The health and weight of the GP were carefully monitored over the following weeks. Animals were deafened two weeks before implantation.

2.8. Cochlear implantation

Using injected anaesthesia as described for ABRs, a post-auricular incision was made to expose the tympanic bulla. The bulla was opened using a 2 mm cutting drill-bit and a cochleostomy performed with a 0.6 mm diamond drill-bit. The polymer-coated electrode was inserted into the cochlea through the cochleostomy (Fig. 1b). Connective tissue was placed around the insertion site to promote sealing