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Effect of the dopant anion in polypyrrole on nerve growth and release of a neurotrophic protein

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ABSTRACT

The dopant anion in polypyrrole plays a critical role in determining the physical and chemical properties of these conducting polymers. Here we demonstrate an additional effect on the ability to incorporate and release a neurotrophic protein – neurotrophin-3. The multi-faceted role of the dopant is critical in ensuring optimal performance of polypyrroles in their use as platforms for nerve growth. In this paper, the effect of changing the co-dopant used in electrochemical polypyrrole synthesis on the compatibility with primary auditory nerve tissue is considered and compared to some of the physical properties of the films. Significant differences in the controlled-release properties of the films were also observed. The ability of the polymers to enhance nerve growth and survival *in vitro* with neurotrophin-3 release was also studied, which is a function of both compatibility with the neural tissue and the ability of the polymer to release sufficient neurotrophic protein to affect cell growth. A small synthetic dopant, *para*-toluene sulphonate, was found to perform favourably in both aspects and ultimately proved to be the most suitable material for the application at hand, which is the delivery of neurotrophins for inner-ear therapies.

1. Introduction

Sensorineural hearing loss is the most common form of hearing loss and involves the loss of sensory hair cells. In severe cases, cochlear implantation is the only option available to restore hearing. However, the loss of hair cells leads to progressive degeneration of auditory neurons (spiral ganglion neurons) and eventual apoptosis of these cells [1,2]. The loss of these spiral ganglion neurons can have detrimental affects on the functioning of the cochlear implant, due to a decrease in the integrity of the nerve–electrode interface [3]. Strategies to prevent the degeneration of the auditory nerve are therefore of interest to improve the function of the cochlear implant.

The application of neurotrophins to the cochlea as a means of promoting survival of spiral ganglion neurons has been investigated over several years. Various neurotrophins, including neurotrophin-3 (NT-3), have been applied to cultured neurons and to the cochlea *in vivo* (for a review of potential clinical applications of neurotrophins in inner-ear therapies, see [4]). Both NT-3 and another neurotrophin, brain-derived neurotrophic factor (BDNF), have been shown to enhance nerve survival when applied either immediately post-deafening [5–7], or after partial neural degeneration has occurred post-deafening [8,9]. Two major challenges remain for therapeutic use of the neurotrophins in the deafened cochlea. Firstly, a safe method is required to deliver the therapeutic proteins into the cochlea that minimises the risk of infection and does not disrupt the function of the cochlear implant, and secondly, the neurotrophins must be delivered for an extended period of time for the rescued nerves to become established. Polypyrrole (PPy) has potential to act as a controlled-release material to provide neurotrophin delivery without disrupting the cochlear implant function, as it could coat the existing platinum electrodes without increasing the impedance, adding additional surgical steps, or introducing additional devices into the ear.

The use of PPy as a matrix for storage and electrically-controlled delivery of drugs has been investigated for several molecules [10–18]. The authors have previously reported the controlled delivery of nerve growth factors, in particular NT-3 [19,20] and brain-derived neurotrophic factor [21,22]. An important consideration in use for the controlled release of molecules is the dopant used during the electrochemical synthesis of PPy films. While often the dopant is the drug or molecule of interest, some systems use a co-dopant to reduce the amount of drug used, or to improve the electrical, mechanical or other physical properties of the polymer [10,11,14,19–24].

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The ability to incorporate and release NT-3 and BDNF is in fact peculiar in that the active molecule to be released should have an overall positive charge (according to its isoelectric point) at the pH values of the receiving media. Despite this, our previous studies have shown that these molecules can be effectively incorporated and released upon electrical stimulation [20–23]. As the bioactive molecule is not the primary dopant, the choice of anionic dopant becomes a critical factor. Given the known effects of the dopant on physical, chemical, electronic and electromechanical properties [25], we have explored the effect on the ability to incorporate and release NT-3. The effect of the dopant used is a complex interplay of the properties induced by the dopant.

It is well documented that the dopant anion in polypyrrole plays a critical role in determining the physical and chemical properties of these conducting polymers. Therefore, in the work described in this paper we try to elucidate the effect of the dopant on the incorporation and release of the neurotrophin NT-3. Six different dopants are used in the synthesis of PPy, namely sodium salts of *para*-toluene sulfonate (pTS), dodecylbenzene sulfonate (DBS), poly (4-styrenesulfonate) (PSS), hyaluronic acid (HA) and chondroitin sulfate (CS) and the ammonium salt of poly(2-methoxyaniline-5-sulfonic acid) (PMAS). The structures of each of these dopants are shown in Fig. 1. In addition, we investigated the behaviour of spiral ganglion neurons (SGN) cultured on the resulting conducting polymer films, with and without incorporated NT-3.

The rationale behind the use of each of these anions varied. The first three dopants, pTS, DBS and PSS, were chosen since they are commonly used as dopant molecules for PPy and have a similar dopant end group ($-SO_3^-$) tethered on small (pTS) to large (PSS) backbones. Previous work using PPy/pTS has been published, both on the material properties [26], and on use of the material for biological applications [27]. Some work using PPy/DBS has been presented in the literature, both for neural applications [28] and for drug-delivery applications (using a biotin-modified pyrrole residue) [29]. PPy/PSS has been studied extensively for biological use, with both non-neural [30–32] and neural [28,33–39] applications investigated. The fourth dopant, PMAS, is a sulfonated and soluble form of the conducting polymer, polyaniline. PMAS has previously been used as a dopant molecule for PPy growth, and the resulting polymer exhibited interesting electrochemical properties [40]. The use of a conducting polymer dopant in the synthesis of another conducting polymer could have interesting implications for incorporation and release of biomolecules. The biocompatibility of PPy/PMAS has also been demonstrated [41], providing sound rationale for the use of this material in further neural cell growth studies.

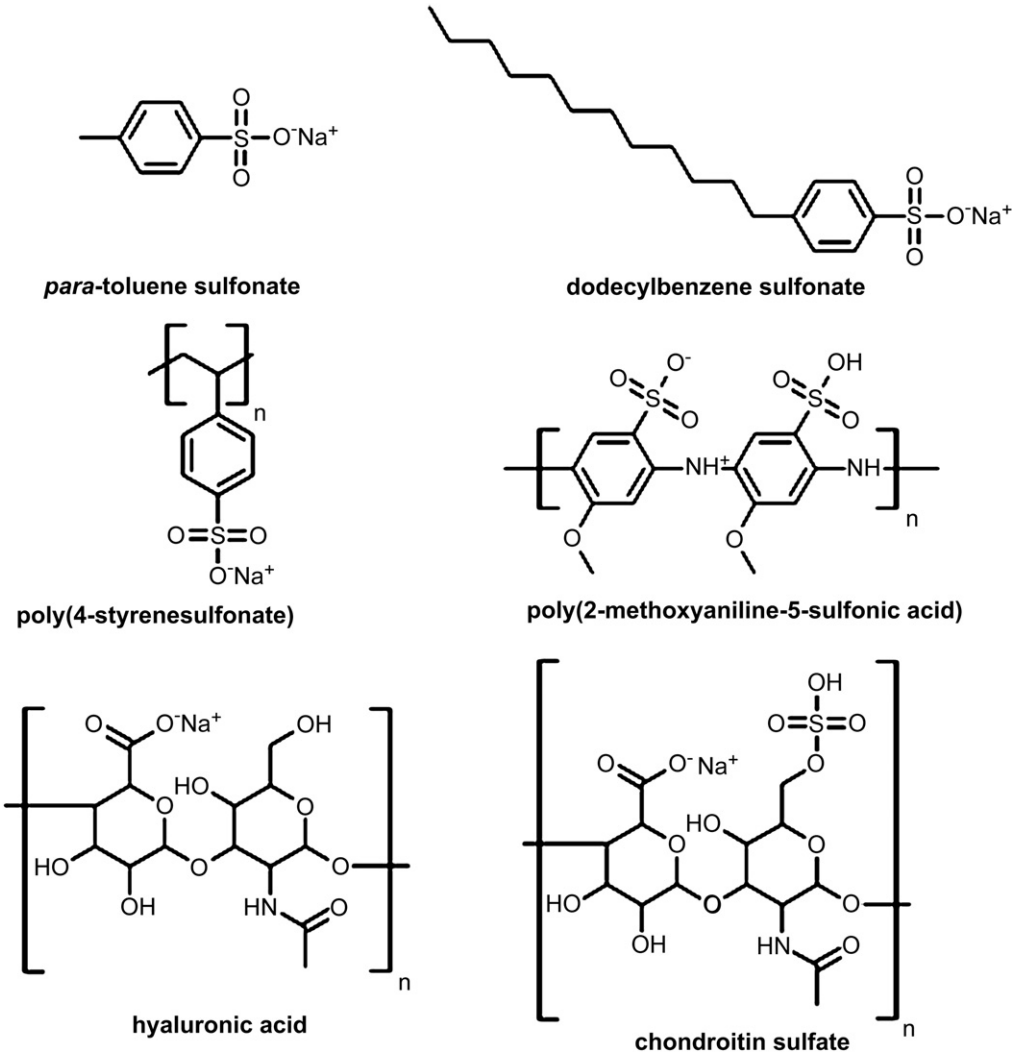

Fig 1. Structures of dopant molecules used in PPy synthesis in this paper.