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Protein adsorption and peroxidation of rat retinas under stimulation of a neural probe coated with polyaniline

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ABSTRACT

For the purpose of investigating the potential use of conducting polymers, i.e. polyaniline (PANi), as electrode coating material for improving the function of neural probes, a PANi-coated platinum (Pt) electrode was prepared by the in situ polymerization method. Protein adsorption was observed by atomic force microscopy/scanning electron microscopy and sodium dodecyl sulfate polyacrylamide gel electrophoresis, as well as quantification. Peroxidation of rat retinas was evaluated by determination of conjugated dienes and PLOOH, which were quantified by UV–visible spectrophotometer and high-performance liquid chromatography. The stability of PANi coating for 6 months was also estimated with an in vitro electrical stimulation system. This revealed that: (1) PANi with regular and compact nanoparticles 20–40 nm in diameter was successfully polymerized on the uncoated platinum electrode surface; (2) the PANi-coated Pt electrode adsorbed fewer retinal fragments and induced less peroxidation than the uncoated platinum electrode; (3) in contrast to the uncoated platinum electrode, the PANi-coated Pt electrode surface tended to aggregate retinal fragments rather than spread them, which may help to reduce inflammation and scar formation in long-term implantation; (4) the PANi coating exhibited excellent properties in terms of the intactness and the stable nanoparticle morphology after 6 months' electrical stimulation, while corrosion occurred on the uncoated platinum electrode after 1 month.

1. Introduction

Current research on microfabrication technology for implantable neural probes has been maturely developed. Processes for fabricating microelectrodes from glass, gold, platinum (Pt), silicon and various polymers are already established [1–4]. However, there still have two factors inhibiting their clinical application. (1) Implantation of stimulating electrodes can produce soft tissue wounds with inflammation, which is a host response to destroy, dilute or wall off the injurious agents and injured tissue. If inflammation persists for more than 2 weeks, it is classified as chronic [5]. The localized defense mechanism generally leads to encapsulation by microglia, astrocytes, endothelia, fibroblasts, etc., and increases the impedance across the electrode to limit the functionality. As a result, a higher input energy could be required and ultimately lead to device failure [6]. Observation of the encapsulation of micromachined silicon devices of different size and cross-section was carried out by Turner et al. [7] and Szarowski et al. [8]. This showed a clear GFAP-positive astrocytes sheath around the insertion site after 4 weeks and an increased number of GFAP-positive stellate astrocytes. This prolonged response has been found to be independent of the device size, geometry and surface roughness, and revealed that implant coating materials with properties of reducing protein adsorption and cell attachment may be critical to long-term functionality. (2) Another factor is the function degradation caused by the corrosion of the noble metal electrode. Pt and its alloys have demonstrated acceptable electrode behavior because of the inertness, biocompatibility and the ability of reducing charge gathering from electrical stimulation. However, Pt electrodes using direct currents to stimulate in the moist environment functioned with far less electrical impedance and toughness than in body surfaces in contact with dry air. In the moist oral cavity, Pt electrodes produced a necrotic tissue response on non-keratinizing tissue even at relatively low current densities of <100 $\mu A\ cm^{-2}$ [9]. For neural and neuromuscular prostheses, where the current densities reach 0.1–0.6 $A\ cm^{-2}$, there can be degradation of the noble metal electrodes and problems with biocompatibility under chronic stimulation [5].

Current work moves towards the improvement of the tissue–electrode interfaces. Approaches in improving the implant–tissue interface include surface modification by electrochemical

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polymerization and covalent immobilization of a biocompatible coating [10]. Ideally, the electrode material would be bioactive in encouraging neurite growth around the electrode as well as preventing the formation of a fibrous capsule. The ability to achieve this is very limited when using metals, but using polymers allows for enhanced bioactivity [6]. There are a few polymers with dual properties of biocompatibility and electrical conductivity, such as polypyrrole and polyaniline (PANi) [11,12]. They exhibited metal-like conductivity and were used as coatings on electrodes rather than as free standing material, owing to their very fragile nature [13,14]. PANi is one of the most promising electrically conductive polymers and has been widely investigated because of its unique properties, including controllable electrical conductivity, environmental stability, and optical and electro-optical properties [12,15]. It is possible to achieve relatively high conductivities within the appropriate fabrication technique. By electrosynthesis, PANi can be coated on inert electrodes such as gold, Pt and aluminium alloy [16]. Many reports present the production of PANi coating by casting [17,18]. Previous studies prepared the synthesized PANi films through modified polymerization. The synthesized PANi films were composed of nanoparticles at 30–50 nm and had much better cell behavior with PC-12 pheochromocytoma cells, such as attachment and proliferation, while the casting PANi film had no such cell behaviour and structure on the nanoscale [19]. Besides PC-12, more evidence showed the ability of PANi and its variants to support cell growth, especially neural cells [20]. Also, it supports the adhesion and proliferation of H9c2 cardiac myoblasts and enhances in vitro neurite extension [21]. A large surface/volume area, controllable surface morphology, wettability, high capacitance and the ability to remain conductive over long periods afford benefits for neural probes. These advances encourage the development of optimized neural electrodes.

Nevertheless, neural electrodes can cause damage to the nerve [22]: first, by the surgical implantation procedure, depending on how much the nerve is separated from the neighbouring structures, accompanied by traumatic interference with the vascular supply. The surgical procedure can damage neighbouring arterial beds and possibly the internal capillary network, as well as the nerve fibres and fascicles, which results in various degrees of functional impairment and intra- or extraneural fibrosis, which interferes with intraneural blood flow [23]. Another potential of damage can be the electrode itself, which produced mechanical restriction on the nerve, but such injury depends on the degree of tissue–electrode contact and its duration. Evidence suggests that overstimulation may either modulate vital ionic and protein gradients, leading to cell death, or generate cytotoxic and apoptotic products by Faradaic reactions at the electrode–tissue interface [24,25]. Besides physical injury, dissolution of metal electrodes, excitotoxicity and dielectric breakdown of the cell membrane also produce neural damage in the cerebral cortex [26]. Most studies examining chronic stimulation injury in the retina have relied on anatomical methods of assessing cytotoxic damage [27,28].

Retina is the eye neurosensorial tissue and is very rich in polyunsaturated fatty acids (PUFA). It is an excellent model for studying electrical stimulation-induced peroxidation because of its high susceptibility to oxidative damage due to the high content of PUFA. The reactive oxygen species induce lipid peroxyl radical formation and initiate lipid peroxidation, which can injure the retina, especially the membranes that play important roles in visual function [29]. Furthermore, biomolecules such as proteins or amino lipids can be covalently modified by lipid decomposition products, including forming Schiff bases with aldehydes or/and activating membrane-bound enzymes [30–32].

Tissue lipid peroxidation during electrical stimulation was detected by Devyatkina et al. [33]. An increase in lipid peroxidation was found to be raised during stimulation. Lipid peroxidation is a complex process which refers to the oxidative degradation of lipids. The process is a self-perpetuating reaction, called a "chain reaction mechanism", involved in the abstraction of a hydrogen atom from a methylene carbon to form a lipid hydroperoxide on PUFA, and rearrangement of the double bonds to form an alkyl radical with a conjugated diene. It continues until meeting termination by free radical scavengers or other free radicals. Breakdown of lipid hydroperoxides leads to the formation of various molecular species and causes cytotoxicity [34]. In ocular tissue, it is correlated to a wide range of disease processes, including cataractogenesis and retinopathy of prematurity [35,36].

In this paper, retinal homogenate was used to evaluate tissue damage from the viewpoint of biochemistry from both phospholipid (PL) peroxidation and protein denaturalization under electrical stimulation of the uncoated Pt electrode with or without PANi coating. The PANi-coated Pt electrode, with a regular and compact nano-PANi-particle surface, was fabricated by in situ polymerization of PANi onto the uncoated Pt electrode surface. Dynamic adsorption of retinal fragments was detected by atomic force microscopy/scanning electron microscopy (AFM/SEM) and was quantified by micro BCA protein assay; peroxidation of rat retinas induced by electrical stimulation from the PANi-coated Pt electrode was evaluated by quantifying the change of conjugated dienes and phospholipid hydroperoxides (PLOOH), especially phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH); changes of retinal proteins from electrode surfaces were observed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Long-term in vitro electrical stimulation of the uncoated Pt electrode with or without PANi coating was also observed by SEM and UV–visible spectrophotometry.

2. Material and methods

2.1. Animals and materials

Sprague–Dawley (SD) rats (220 ± 30 g) were purchased from the Animal Center of Fudan University, and the study design was approved by the Ethical Committee of Animal Experiments of Shanghai Jiao Tong University.

Aniline was purchased from Aldrich (St Louis, MO). $HCLO_4$ and ammonium peroxydisulfate ($(NH_4)_2S_2O_8$) were purchased from SCRC (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and used as received. A micro BCA protein assay reagent kit and coomassie blue dye were purchased from Pierce (Rockford, IL, USA). l-α-Phosphatidylcholine was purchased from Sigma (St Louis, MO, USA). 2, 6-di-*tert*-butyl-4-methyl-phenol (BHT) and SDS were purchased from Acros (Geel, Belgium). The Pt wire, with purity 99.9%, was purchased from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (Shanghai, China).

2.2. Preparation of retinal homogenate

After being dark-adapted, SD rats were anesthetized with intraperitoneal injections of pentobarbital sodium. Isolation of retinas was carried out under 4 °C. The obtained retinas were pre-washed in ice-cold solution of 0.9% sodium chloride, and were then homogenized in the same solution in a FLUKO Superfine Homogenizer (F6/10, Germany) at 10,000–15,000 rpm [37]. The content of total protein was adjusted to 0.5 mg ml^{-1} before use [38].

2.3. Fabrication of the PANi-coated Pt electrode

The PANi-coated Pt electrode was prepared by the in situ polymerization method according to previous studies [19,39,40]. The