Materials Science and Engineering C 54 (2015) 176–181

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Two approaches to the model drug immobilization into conjugated polymer matrix

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ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 10 February 2015
Accepted 7 May 2015
Available online 8 May 2015

Keywords:
Conjugated polymers
Polypyrrole
Drug immobilization
Controlled release
Quercetin
Ciprofloxacin

ABSTRACT

The purpose of this study is to develop biocompatible and conducting coating being carrier of biologically active compounds with the potential use in neuroprosthetics. Conducting polypyrrole matrix has been used to immobilize and release model drugs, quercetin and ciprofloxacin. Two routes of immobilization are described: drugs have been incorporated in the polymer matrix in the course of the electropolymerization process or after polymerization, in the course of polymer oxidation. Using UV/Vis spectroscopic detection we demonstrate that both immobilization approaches display different drug-loading efficiencies. In the case of ciprofloxacin, drug incorporation following synthesis is a more efficient immobilization approach (final drug concentration: 43.3 (±9.5) μM/cm^2), while for quercetin the highest loading is accomplished by drug incorporation during synthesis (final drug concentration: 29.1 (±5.9) μM/cm^2). The process of drug incorporation results in the variation of surface morphology with respect to the method of immobilization as well as the choice of drug. The results prove that electrochemical methods are efficient procedures for making multifunctional polymer matrices which might be perspective bioactive coatings for implantable neuroprosthetic devices.

1. Introduction

Implantable neuroprosthetic devices are versatile and powerful tools used to record electrophysiological activity in neural tissue [1], restore neural functions [2,3] or treat long-term neurological disorders, e.g. epilepsy [4,5]. However, it is known that metallic neural electrodes, even those made of titanium and its alloys, may be a source of allergic reactions, inflammation and infections [6]. For that reason, intensive research has been conducted to develop biocompatible coatings preventing implant-associated health hazards [7]. The optimal interface material has to fulfill several requirements including biocompatibility, conductivity, low interface impedance and high charge storage capacity [8]. It would be beneficial if the interface could also act as a reservoir of anti-inflammatory or antibiotic drugs [9]. These features come together in conjugated polymers which, due to their conductivity and biocompatibility, could be used as materials for biomedical engineering [10]. Conjugated polymers have been successfully applied as biosensors [11], molecular scaffolds [12] and bioactuators [13]. Due to their ion-exchange properties, these polymers are able to immobilize and release biologically active molecules in a highly controlled way [14].

In vitro characterization of cell responses proved that conjugated polymers provide more favorable environment for cell attachment and growth than bare metal implant [15]. Besides, conducting polymers being carriers of neurotrophins are able to stimulate neurite growth of target cells [16]. Cui et al. [17] showed that the surface modification of a neural microelectrode with polypyrrole combined with fibronectin fragments (SLPF) and nonapeptide CDPGYIGSR is conducive for the attachment of neuroblastoma and glial cells. The rough and fuzzy morphology of the coating helps to lower the impedance of the electrodes and improve the signal transport. Stauffer et al. [18] demonstrated the application of controlled release of neurotrophins from a conjugated polymer microelectrode in the modulation of local neural activity. The release of inhibitors of NMDA and AMPA-type glutamate receptors from electrodeposited polypyrrole coating was realized by the application of brief voltage pulses (duration from 200 ms to 4 s, magnitude from 1.0 V to 5.0 V). After the released neurochemicals achieved locally effective concentrations, they caused the inhibition of evoked synaptic currents in neurons for several seconds. This technique enables achieving advanced patterned chemical modulation of neuronal circuits and can be applied for both in vitro and implantable neural microelectrode arrays.

Several approaches to drug immobilization into conducting matrices have been described in the literature including drug incorporation during synthesis [19–21] and drug incorporation following synthesis [22–24]. The immobilization during synthesis relies on ionic interactions between negatively charged drug and positively charged growing polymer chain (co-doping process) [19]. The in-situ method can be applied to immobilize positively charged and neutral drugs — this immobilization approach is based on the combination of hydrophobic and electrostatic interactions between drug, anionic primary dopant and

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http://dx.doi.org/10.1016/j.msec.2015.05.017
0928-4931/

growing polymer chain together with physical entrapment [20,21]. The main disadvantage of this method is the possibility of the interference between biomolecules and growing polymer chain. Drug immobilization following the synthesis is a more sophisticated method comprising three steps: polymerization of conducting polymer matrix followed by the process of its reduction (to get rid of primary dopant) and oxidizing treatment in the solution of negatively charged drug (to immobilize biomolecules) [22]. The mechanism of positively charged drug immobilization relies on the ionic interactions between bioactive molecules (e.g. dopamine) and large immobilized anions such as poly(styrene sulfonate) being a primary dopant [19]. Using β-cyclodextrins as dopant is a novel approach to immobilize neutral drugs (e.g. N-methylphenothiazine) in conducting polymer matrix [24]. Cyclic voltammetry and chronoamperometry are both proven to be successful methods of drug release for immobilization procedures [14].

Amino glycosides and quinolones are the most widely acceptable antibacterial agents used in local delivery systems, especially for chronic osteomyelitis [25]. Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid) is a fluoroquinolone derivative frequently prescribed for treating several bacterial infections caused by Gram-positive and Gram-negative bacteria [26,27]. The mode of action of ciprofloxacin involves the inhibition of the enzyme activity necessary for replication, transcription, repair and recombination of bacterial DNA, mainly DNA gyrase and topoisomerase IV [27,28]. Research performed on coaxial conducting polymer fibers loaded with ciprofloxacin confirmed that drug molecules subjected to electrochemical immobilization and electrically-triggered release retained their antibacterial properties [29]. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) belongs to the group of flavonoids and can be found in numerous vegetables and fruits [30]. It is a potent oxygen free radical scavenger and has substantial anti-inflammatory, anti-cancer, anti-ulcer, anti-allergic, antiviral and antibacterial activities [31–33]. These properties can be attributed to the ability of quercetin to inhibit lipopolysaccharide-induced pro-inflammatory cytokine production, reduce the release of TNF-α and IL-1β, thereby alleviating inflammatory responses [34]. To the best of our knowledge, the immobilization of quercetin into conducting polymer matrix has not been described in the literature so far.

The aim of this work is to study the capability of polypyrrole matrix to immobilize and release two model drugs, quercetin and ciprofloxacin. Two routes of immobilization are described in this work: drugs are incorporated in the polymer matrix in the course of the electropolymerization process or after polymerization, in the course of polymer oxidation. The effectiveness of immobilization and further release are compared by means of UV/Vis spectroscopy, while the information about the surface morphology is provided through scanning electron microscopy.

2. Materials and methods

2.1. Materials

Pyrrole, Py (Aldrich, 98%) was purified by distillation under reduced pressure. Ciprofloxacin, $C_{17}H_{18}FN_3O_3$ (FLUKA, 98%), quercetin, $C_{15}H_{10}O_7$ (Sigma-Aldrich, 95%), potassium chloride (Avantor, analytical grade), dipotassium hydrogen phosphate (Avantor, analytical grade), and potassium dihydrogen phosphate (Avantor, analytical grade) were used as received. Grade 1 ($R > 10\ M\Omega \cdot cm^{-1}$) deionized water was employed as solvent for all prepared solutions.

2.2. Instrumentation

Immobilization and release processes were carried out in a standard three-electrode setup, employing a platinum foil working electrode (1 cm^2), Ag/AgCl reference electrode and glassy carbon counter electrode. Electrochemical measurements were performed using a CH Instruments 620a Electrochemical Workstation. Spectroscopic measurements were performed using a Hewlett Packard 8453 UV/Vis Diode Array Spectrophotometer. Surface morphology was analyzed with the use of a Phenom Pro-X scanning electron microscope.

2.3. Methods

2.3.1. Drug incorporation during the process of electropolymerization

The in-situ drug immobilization technique involved the electrochemical polymerization of monomer (0.1 M Py) in aqueous suspension with pH = 7 containing supporting electrolyte (1 M KCl) and the model drug (6 mM Que or 7 mM Cpf, respectively). Ciprofloxacin is a zwitterionic molecule with pKa of 6.09 and 8.74 [35]. It was found that at neutral pH more than 85% of Cpf is present in its zwitterionic form [36]. Deposition of the composite layer was achieved during 25 potential cycles in the range of −0.8 V ÷ 0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V/s.

2.3.2. Drug incorporation following the process of electropolymerization

The ex-situ drug immobilization comprised the electrochemical polymerization of Py (0.1 M) in 1 M KCl aqueous solution, followed by the dedoping of the polymer layer and oxidative immobilization of drugs. Polymerization was realized via 25 potential cycles in the potential range of −0.8 V ÷ 0.8 (vs. Ag/AgCl) V, at a scan rate of 0.1 V/s. Dedoping of the polymeric deposit was performed in 1 M KCl aqueous solution, at a constant working electrode potential of −0.7 V (vs. Ag/AgCl) over 10 min. Immobilization of model drugs featured potentiostatic oxidation of the polymer at 0.6 V (vs. Ag/AgCl) in 6 mM Que or 7 mM Cpf aqueous suspension, respectively, for the duration of 10 min.

2.3.3. Drug release studies

Prior to the investigation of drug release, the electrodes covered with PPy/Cpf and PPy/Que were soaked in phosphate buffer saline solution (PBS, pH = 7) comprising 0.15 M KCl, 0.006 M K_2HPO_4 and 0.001 M KH_2PO_4 for 10 min. The goal of such treatment was to remove any loosely attached molecules of drugs and unreacted monomer.

The release of model drugs from PPy/Cpf and PPy/Que composites submerged in PBS was realized in a 2 mm quartz cuvette (Hellma Analytics, type no. 100-QS). The electrodes were positioned beyond the optical path to allow in situ monitoring of the process by time-resolved UV/Vis spectroscopy. Electrically-triggered (active) drug release was performed by applying a reduction potential of −0.5 V (vs. Ag/AgCl), whereas spontaneous (passive) drug release was carried out under open circuit conditions. UV/Vis spectra were acquired every 2 min and the evolution of absorption at 322 nm for Cpf and 374 nm for Que was exploited for the determination of drug concentration in solutions. Concentrations of Que and Cpf were determined through the use of the calibration curve method. Drug concentrations achieved during release were expressed in respect to the active area of the electrode covered with a polymer layer (1 cm^2). The choice of neutral pH was caused by the necessity to meet the physiological conditions. For both drugs the sink condition was maintained [37–39].

3. Results and discussion

3.1. Synthesis of PPy/Cpf and PPy/Que

According to the literature reports, there are two main methods of drug immobilization into conducting matrices, namely drug incorporation during and following the process of electrochemical polymerization. Both approaches have their advantages and disadvantages and can differ in performance depending on a chemical structure of immobilized biomolecule. Due to the ionic nature and hydrophobic character of drugs, their immobilization can rely on ionic interactions or physical entrapment. Therefore, both immobilization methods are expected to exhibit different drug-loading efficiencies caused by the zwitterionic character of ciprofloxacin and neutral-charge form of quercetin (Scheme 1).