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Advancing the delivery of anticancer drugs: Conjugated polymer/triterpenoid composite

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ABSTRACT

Exemplifying the synergy of anticancer properties of triterpenoids and ion retention qualities of conjugated polymers, we propose a conducting matrix to be a reservoir of anticancer compounds. In this study, poly(3,4-ethylenedioxythiophene), PEDOT, based matrix for electrically triggered and local delivery of the ionic form of anticancer drug, oleanolic acid (HOL), has been investigated. An initial, one-step fabrication procedure has been proposed, providing layers exhibiting good drug release properties and biological activity. Investigation of obtained systems and implementation of modifications revealed another route of fabrication. This procedure was found to yield layers possessing a significantly greater storage capacity of OL^-, as evidenced by the 52% increase in the drug concentrations attainable through electro-assisted release. Examination of the biological activity of immobilised and released OL^- molecules proved that electrochemical treatment has negligible impact on the anticancer properties of OL^-, particularly when employing the three-step procedure, in which the range of applied potentials is limited. PEDOT/OL^- composite has been demonstrated to be a robust and cost-effective material for controlled drug delivery.

1. Introduction

Chemotherapy is one of the most widely utilised procedures for treating cancer [1]. Despite its many merits, the application of this treatment is limited by severe toxic side effects of anticancer drugs on healthy tissues [2,3]. Efforts are being made to tackle this issue by developing more benign drugs [4]. Recent reports, however, show that it is also possible to overcome this challenge by exploiting the potential of local drug delivery systems (DDS) for the deployment of anticancer agents [5].

The highlight of a localised DDS approach is the possibility of implanting drug-releasing devices directly at the tumour site. Proceeding this way, it is possible to minimise both systemic exposure and side effects of chemotherapy [6]. Several approaches to the development of such systems have heretofore been reported, utilising chitin microparticles [7], biodegradable polymeric microspheres [8], poly(D,L-lactide-co-glycolide) wafers [9], poly[bis(p-carboxyphenoxy)propane-sebacic acid] copolymer discs [10] as well as other, intravenous delivery systems, based on nanoparticles and polymers [11,12]. All of the above share a common mechanism of drug delivery – the spontaneous release of bioactive molecules from the matrix upon its bio-assisted decomposition.

Conjugated polymers, possessing ion-exchange properties, are considered promising materials for use as drug reservoirs in drug delivery systems [13]. In contrast to the physical entrapment [14], conjugated polymers allow controlled, reversible electrostatic immobilisation. The mechanism of this process relies on the fact that conducting polymers, depending on their oxidation state, undergo a charging–discharging process and adopt positive or negative charges. These charges draw ions of opposite charge into the polymeric matrix, binding them via Coulomb interactions. Therefore, they are able to immobilise anionic drugs during oxidation (doping) and release them in the process of reduction (dedoping).

The controlled immobilisation/release mechanism is highly desired, however, the development of implantable drug delivery systems necessitates all of the device constituents to be fully

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biocompatible. Although biocompatibility is not inherent to conjugated polymers, some among them, such as polypyrrole [15] and poly(3,4-ethylenedioxythiophene) (PEDOT) [16], exhibit this trait.

Consequently, conducting polymers have been used in the design of novel electrochemical drug release devices [17]. Lira et al. [18] described a polyaniline-based device able to release safranin as a result of electric pulse. The amount of released drug was found to be dependent on the oxidation state of polyaniline which was modified through the application of potential. Valdes-Ramirez et al. [19] developed a microneedle-based drug delivery system, in which controlled release of multiple therapeutic agents was realised by the use of a polypyrrole (PPy) nanoactuator. PPy in its reduced state obstructed the flow of the therapeutic solution through the needle; the oxidation of PPy was followed by its contraction and the facilitation of flow. Nevertheless, a significant issue connected with the use of conducting polymers as drug delivery devices is the method for altering the redox state of the polymer. The majority of reported, electrically triggered delivery systems, based on conjugated polymers, rely on percutaneous electrodes to deliver electric current. The necessity of ensuring physical electrical contact with polymer matrix significantly hindered the practical use of conducting polymers. This issue, however, has been resolved by Gao et al. [20], who found an effective way to change the redox state of polymer remotely. Dexamethasone-saturated polypyrrole platforms were designed to be sensitive to local, pulsatile electromagnetic field. The remote control of oxidation state of the polymer provided the framework for an electrically-triggered device that can be implanted into the body without requiring any physical contact with the surroundings for operation.

Superior chemical and electrochemical stability is the primary highlight of PEDOT, when compared to other conjugated polymers [21]. Heretofore, there have been several reports concerning the use of PEDOT matrices for electrochemical immobilisation and electrically triggered release of biomolecules. These have utilised pure PEDOT [22], as well as its composites with carbon nanotubes [23] and polystyrene sulphonate [24]. In each case, doping/dedoping of PEDOT was exploited for controlled drug release, evidencing the versatility of this mechanism of immobilisation [25].

Triterpenoids are one of the most promising groups of anticancer compounds. These compounds are secreted by living tissues of numerous higher plants, granting them exceptionally good availability. To exemplify, oleanolic acid (HOL) can be isolated from more than 1600 plants species, including common flora, such as olive, mistletoe, lavender, rosemary, clove and many others [26]. The prime highlight of HOL is its anticancer activity in various stages of tumour development, including inhibition of tumour promotion, invasion and metastasis. This quality has been reported in a number of cell lines, including HeLa [27], L1210, K562 and HL-60 [28], HepG2, Hep3B, Huh7 and HA22T [29]. Furthermore, numerous tests have evidenced the anti-diabetic [30,31], antiviral [32,33], antibacterial [34], antifungal [35], anti-inflammatory [36–39], anti-allergic [39], wound-healing [40], nephroprotective [41], diuretic [42] and hepatoprotective [43] activity of oleanolic acid. Whereas its anticancer activity is of prime importance, this impressive array of secondary, beneficial qualities may aid in combating possible post-chemotherapy complications.

Encouraged by these superior traits, numerous derivatives of oleanolic acid have been synthesised and were reported to exhibit anticancer activity against many cancer cell lines, e.g. PC3, A549, BGC-823 [44], MCF-[44,45], KB, HeLa [45], Hep-G2 [46], CCRF-CEM, CCRF-VCR1000, CCRF-ADR5000 [47]. Therefore, should the systems designed for delivery of HOL achieve success, it would be valuable to explore triterpene-based DDSs.

In this study we describe a robust and cost-effective method to fabricate PEDOT/OL^- composite layers for drug delivery. Two procedures of immobilisation have been proposed: immobilisation during the process of polymerisation and post-polymerisation modification. The latter was found to yield layers possessing a significantly greater storage capacity of OL^-. Examination of the biological activity of immobilised and released OL^- evidenced that electrochemical treatment has negligible impact on the anticancer properties of OL^-, particularly when employing the three-step procedure, in which the range of applied potentials was limited. PEDOT/OL^- composite was found to exhibit good properties in terms of controlled release, along with maintaining biological activity of the embedded drug, making it a promising candidate for further development and commercial application.

2. Materials and methods

2.1. Materials

Diethyl ether (Chempur, analytical grade), sodium hydroxide (Chempur, analytical grade), ethyl alcohol (Chempur, analytical grade), hydrochloric acid (Chempur, analytical grade) were used to isolate oleanolic acid.

Trichloroacetic acid 99% (SigmaAldrich), acetic acid 99% (Chempur), sulphorhodamine dye (SigmaAldrich), Tris buffered saline (SigmaAldrich), aqua pro injectione (Baxter), HeLa human cell line (ECACC), KB cell line (human nasopharynx carcinoma) (ECACC), A-549 cell line (ATCC), Streptomycin (Polfa), Penicilinum (Polfa), PBS buffer tablets (SigmaAldrich), Fetal Bovine Serum (FBS) (BecktonDickinson), dimethyl sulphoxide (DMSO) – (SigmaAldrich), Dulbecco's Modified Medium (DMEM) – (Sigma Aldrich), RPMI-1640 Medium (SigmaAldrich) were used to perform anticancer activity tests.

3,4-ethylenedioxythiophene (EDOT) (Sigma–Aldrich, >97%), lithium perchlorate (Sigma–Aldrich, >95%, ACS grade), potassium chloride (Avantor, analytical grade), dipotassium hydrogen phosphate (Avantor, analytical grade), potassium dihydrogen phosphate (Avantor, analytical grade), ethyl alcohol 99.8% (Avantor) and sodium hydroxide (Avantor, analytical grade) were used as received. Grade 1 ($R > 10\ M\Omega\ cm^{-1}$) deionised water was employed as solvent for all prepared solutions.

2.2. Instrumentation

Büchi apparatus was used to determine the melting point of oleanolic acid. Varian Gemini 300 VT apparatus was used to record ^1H, ^{13}C and DEPT spectra of isolated oleanolic acid.

Laminair Heraeus Class II, Microplate reader Tecan sunrise, Thermomixer eppendorf, CO_2 Incubator New Brunswick 170R, Ultra Low Temperature Freezer New Brunswick C340 Premium, Centrifuge Sigma 1–6, Laboratory Analytical Scale (Radwag), Centrifuge Z 326K (Hermle) were used to perform anticancer activity tests.

Immobilisation 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 a glassy carbon counter electrode, therefore, all potentials in this work are reported in relation to Ag/AgCl. Electrochemical measurements were performed on a CH Instruments 660c Electrochemical Workstation. Spectroscopic measurements were executed on a Hewlett Packard 8453 UV–Vis Diode Array Spectrophotometer.

2.3. Methods

2.3.1. Preparation of oleanolic acid

Oleanolic acid was isolated from waste-product obtained during production of mistletoe extract. This waste product, in a form

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