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Conductive polymer-based bioelectrochemical assembly for *in vitro* cytotoxicity evaluation: Renoprotective assessment of *Salvia officinalis* against carbon tetrachloride induced nephrotoxicity

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ABSTRACT

Background: The rise of organic electronics represents one of the most prominent technological developments of the last two decades, with its interface with biological systems highlighting new directions of research. The "soft" nature of conducting polymers renders them unique platforms for cell-based microdevices, allowing their implementation in drug discovery, pharmaceutical effect analysis, environmental pollutant testing *etc*.

Methods: Cellular adhesion, proliferation and viability experiments were carried out to verify the biocompatibility of a PEDOT conductive polymer surface. Cyclic voltammetry was employed for estimating the electrocatalytic activity of the renal cell/electrode interface. The nephrotoxicity agent CCl_4 and the medicinal plant *Salvia officinalis* were used on the proposed assembly. Renal cell viability was also assayed through the MTT assay.

Results: Renal cells were able to adhere and proliferate on the conducting polymer surface. Electrochemical responses of the polymer exhibited good correlation with cell number and CCl_4 concentration. Amelioration of the CCl_4-induced renotoxicity by co-incubation with *Salvia officinalis* extract was demonstrated by both the MTT assay and the electrode's capacitance.

Conclusions: A conducting polymer-based bioelectrochemical assembly was established for *in vitro* mammalian cytotoxicity/cytoprotection assessment, employing renal cell monolayers as the primary transducers for signal generation and biological sensing.

General significance: The knowledge on PEDOT mammalian cell biocompatibility and possible applications was expanded. The proposed interdisciplinary approach connects soft electronics with biology and could provide a useful tool for preliminary crude drug screening and bioactivity studies of natural products or plant extracts *in vitro*.

1. Introduction

The rise of organic electronics represents one of the most prominent technological developments of the last two decades, with its interface with biological systems being a dynamically emerging field that highlights new directions of research. Apart from their general advantages like low cost of materials, tunable electronic or optical properties, easy process ability, light weight, low environmental impact and potential for low cost [76,96], organic electronics are gaining momentum as remarkable transducers of biological events. The soft mechanical properties of many organics offer better compatibility with delicate biological tissues and cells than traditional electronic materials, while their ability to conduct ions in addition to electrons and holes opens up a new communication channel with biology [57,79].

Conducting polymers (CPs), typically employed in organic electronics, are polymeric materials, organic in nature that hold unique properties such as electrical conductivity, high electron affinity, low ionization potential and redox activity [9,94]. Additionally, their

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plasticity, which allows them to act as excellent materials for immobilization of biomolecules, renders them unique biosensor platforms [8,38], while their ability to act as both sensing elements and transducers during biological recognition events, simplifies biosensor designs [2,9].

Among CPs, Poly (3,4ethylenedioxythiophene) (PEDOT) has become an established electronic material, holding the highest market share for conducting polymers in the capacitor market today [33]. PEDOT, which was found to be almost transparent in thin oxidized films, exhibits not only a high conductivity but also high stability in oxidized state and has been considered as perhaps the most stable conducting polymer currently available [26,42]. Due to its biocompatibility and similarity to biological compounds, PEDOT has been regularly used in bioengineering and biosensing applications [16,50,70,88]. PEDOT films have been widely demonstrated to support the adhesion and proliferation, among others, of fibroblasts [24], neural [41], neuroblastoma [6] and epithelial [14] cells.

Cell-based biosensors combine intact living cells with sensors or transducers for monitoring physiological changes in various cell types caused by internal or external stimuli [17,98]. Because of the complex nature of their biorecognition elements (whole living cells), they naturally encapsulate composite sensor arrays, providing a wide range of sensing capabilities and thus are expected to respond optimally to bioactive analytes [67]. Cell-based microdevices, including cell-based biosensors, are able to assess many cellular responses, such as adhesion, proliferation, integrity, migration, differentiation and signaling [25,36,43], in a manner that allows their implementation in drug discovery, pharmaceutical effect analysis, clinical practice, environmental pollutant testing, food safety *etc*. [11,30,54].

Plants have been used for their therapeutic properties for millennia [91], while natural products have long been served as critical stimuli for drug design [89]. Drug discovery from natural sources is an intrinsically complex process that necessitates highly integrated interdisciplinary research approaches [44]. Historically, drug screening relies principally on animal tests which are expensive, time consuming, low throughput and ethically provoking [5]. These limitations have stimulated continuous research on developing cell-based platforms that can provide *in vivo* biological information and thus reduce the number of animal tests, accelerating the drug discovery process [106]. Literature search on cell-based electrochemical biosensors that have been used for studying the effect of plant extracts [4,53,85], or their bioactive constituents [35,61,74,100] on mammalian cells, reveals only a fair number of reports. Based on the recent revitalization of interest in drug discovery from natural sources [7], the developments on biomaterials for biosensing applications [72,101] and the need to apply high throughput screening technologies in bioactivity detection of plant extracts [45], biosensing approaches are projected to increase.

In this study, a conducting polymer bioelectrochemical assembly was established for *in vitro* mammalian cytotoxicity/cytoprotection assessment, employing renal cell monolayers as the primary transducers for signal generation and biological sensing. Motivated by pharmacological interest in bioactive natural product-related assays, our aim was to develop an electrochemical cell-based approach that could provide cell type specific insight on the biological activity of crude drugs or their bioactive constituents.

Since PEDOTs biocompatibility has been proven with numerous cell lines, the objective of this study was to explore PEDOTs compatibility with kidney fibroblasts and fabricate an electrochemical biosensor platform able to reflect the renal cell homeostasis. For this purpose, we investigated cell adhesion, proliferation and viability indicators on kidney cells cultured on PEDOT electrode surfaces, along with the assessment of the electrodes' electrochemical state using their post-cell adhesion areal capacitance as a figure of merit for cell monolayer structural/functional status. Furthermore, the model renotoxic agent carbon tetrachloride and the widely studied medicinal plant *Salvia officinalis*, were used for the functional evaluation of the proposed assembly as a renotoxicity and renoprotection assay. To the best of our best knowledge, this is the first study describing a bioelectrochemical tool that incorporates mammalian cells, namely a cell based biosensor, with a conducting polymer for voltammetrically assaying the bioactivity of a medicinal plant extract.

2. Experimental

2.1. Materials and reagents

Dulbecco's Modified Eagle medium (DMEM), phenol red-free DMEM, L-glutamine, penicillin-streptomycin antibiotic mixture, 0.25% trypsin-EDTA solution and sodium pyruvate were purchased from Biochrom AG (Berlin, Germany). Fetal bovine serum (FBS) and Propidium iodide (PI) were purchased from Invitrogen (Massachusetts, USA). Ethidium bromide was obtained from Thermo Fisher Scientific (ACROS Organics™ brand) (Waltham, MA, USA). Carbon tetrachloride (CCl_4, PubChem CID: 5943) Neutral Red (NR), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Acridine Qrange (AO), calf thymus DNA and all other reagents, unless otherwise specified, were purchased form Sigma-Aldrich (St. Louis, MO, USA). All aqueous solutions were prepared with distilled water.

2.2. Cell culture

Vero cells (continuous African green monkey kidney cell line/*Cercopithecus aethiops*/ATCC CCL-81, originally provided from LGC Promochem (Teddington, UK) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 1% L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, under standard cell culture conditions (5% CO_2, 95% humidity, 37 °C). Cells were maintained routinely in 75cm^2 cell culture flasks, media were replenished with fresh medium every other day and cells were subcloned through trypsinization (0.25% trypsin, 0.02% EDTA) upon 80%–90% confluency. Cell numbers were determined by hemocytometer.

2.3. Electrode sterilization and cell seeding

PEDOT screen-printed electrodes (SPEs) from Dropsens® (Llanera, Spain) (DRP-P10) were sterilized with 70% ethanol (EtOH) in phosphate buffered saline (PBS) for a few seconds and then washed three times with sterile PBS. A sanitized polystyrene cylinder tube (6 mm height, 4 mm inner diameter) was placed on top of the PEDOT working electrode, forming a cell culture microwell that can hold up to 60 μl of culture medium and defining the cell growth surface. The resulting effective seeding surface area of each electrode was 0.126 cm^2. A schematic representation of the culture setup can be seen in Fig. 1A. Prior to cell seeding, each PEDOT electrode was hydrated in DMEM for 1 h under standard cell culture conditions (5% CO_2, 95% humidity, 37 °C) to saturate the surface with growth medium and improve cell attachment.

For electrode seeding, hydration medium was replaced with 50 μl cell suspension of Vero fibroblasts (passages 129 to 135), adjusted to desired concentrations with DMEM. The cells were seeded at successive densities from 1×10^4/cm^2 to 12×10^4/cm^2 and were allowed to attach (3 h) and proliferate (24 h) under standard cell culture conditions (DMEM/10% FBS, 5% CO_2, 95% humidity, 37 °C), before further analysis. The cell monolayers on the PEDOT electrodes were regularly inspected under phase contrast microscopy, with an inverted microscope equipped with phase contrast illumination (A. Krüss - MBL3200). Electrodes from the designated time points or exposure parameters were randomly selected and harvested aseptically for performing the biochemical and electrochemical measurements.