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Abstract

Poly(3,4-ethylenedioxythiophene) (PEDOT) is an organic conducting polymer that has been the focus of significant research over the last decade, in both energy and biological applications. Most commonly, PEDOT is doped by the artificial polymer polystyrene sulfonate due to the excellent electrical characteristics yielded by this pairing. The biopolymer dextran sulphate (DS) has been recently reported as a promising alternative to PEDOT: PSS for biological application, having electrical properties rivaling PEDOT: PSS, complimented by the potential bioactivity of the polysaccharide. In this work we compared chemical and electrochemical polymerisations of PEDOT: DS in terms of their impact on the electrical, morphological and biological properties of the resultant PEDOT: DS films. Post-growth cyclic voltammograms and UV-Vis analyses revealed comparable redox behaviour and absorbance profiles for the two synthesis approaches. Despite good intrinsic conductivity of particles, the addition of chemically produced PEDOT: DS did not markedly enhance the bulk conductivity of aqueous solutions due to the lack of interConnectivity between adjacent PEDOT: DS particles at achievable concentrations. Scanning electron microscopy revealed significantly greater roughness in films cast from chemically produced PEDOT: DS compared to electropolymerised samples, attributable to the formation of solution phase nanoparticles prior to casting. In cell studies with the L929 cell line, electrochemical polymerisation of PEDOT: DS afforded better integrity of resultant films for surface seeding, whilst chemically polymerised PEDOT: DS appeared to localised at the proliferating cells, suggesting possible applications in drug delivery.

Keywords

chemical, electrochemical, synthesis, sulphate, comparison, pedot, application, bio, dextran

Disciplines

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A Comparison of Chemical and Electrochemical Synthesis of PEDOT:Dextran Sulphate for Bio-Application

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ABSTRACT

Poly(3,4-ethylenedioxythiophene) (PEDOT) is an organic conducting polymer that has been the focus of significant research over the last decade, in both energy and biological applications. Most commonly, PEDOT is doped by the artificial polymer polystyrene sulfonate due to the excellent electrical characteristics yielded by this pairing. The biopolymer dextran sulphate (DS) has been recently reported as a promising alternative to PEDOT:PSS for biological application, having electrical properties rivaling PEDOT:PSS, complimented by the potential bioactivity of the polysaccharide. In this work we compared chemical and electrochemical polymerisations of PEDOT:DS in terms of their impact on the electrical, morphological and biological properties of the resultant PEDOT:DS films. Post-growth cyclic voltammograms and UV-Vis analyses revealed comparable redox behaviour and absorbance profiles for the two synthesis approaches. Despite good intrinsic conductivity of particles, the addition of chemically produced PEDOT:DS did not markedly enhance the bulk conductivity of aqueous solutions due to the lack of interconnectivity between adjacent PEDOT:DS particles at achievable concentrations. Scanning electron microscopy revealed significantly greater roughness in films cast from chemically produced PEDOT:DS compared to electropolymerised samples, attributable to the formation of solution phase nanoparticles prior to casting. In cell studies with the L929 cell line, electrochemical polymerisation of PEDOT:DS afforded better integrity of resultant films for surface seeding, whilst chemically polymerised PEDOT:DS appeared to localised at the proliferating cells, suggesting possible applications in drug delivery.

INTRODUCTION

Since the 1977 discovery of polyacetylene by Heeger, MacDiarmid and Shirakawa¹, the field of conductive polymers has expanded to include a wide array of polymers². Polypyrrole, polyaniline and poly(3,4-ethylenedioxythiophene) (PEDOT) in particular have attracted significant interest as candidates for application in biological systems³. In a recent review examining the applications of conductive polymers, Balint, Cassidy and Cartmell highlighted the importance of dopant selection as a key factor in tailoring conductive polymers to specific

applications⁴. Most often, PEDOT is doped using polystyrene sulfonate (PSS), an artificial polymer selected for the excellent electrical properties of the PEDOT:PSS system. In biological applications however, biocompatibility and bioactivity are paramount, and conductive polymers can benefit from dopants that balance the needs for electrical and biological performance. Recently, Harman *et al.* identified dextran sulphate (DS), a sulfonated polysaccharide, as a promising biological dopant for PEDOT⁵. In that work, PEDOT:DS was chemically synthesised in high yield and shown to be both highly processable and minimally toxic towards the fibroblast cell line L929. In another study by Molino *et al.* PEDOT was electrochemically doped with alginate, chondroitin sulphate and DS⁶. By electrochemically polymerising PEDOT directly onto a quartz microbalance surface the authors probed the cell attachment capacity of the materials, finding that PEDOT:DS supported better cell attachment than PEDOTs doped with either alginate or chondroitin sulphate. This was related to differences in binding capacities for important cell adhesion proteins such as fibronectin⁷. In the present study we further examine the potential of this new material with particular attention to the relative benefits of chemical and electrochemical synthesis modes.

EXPERIMENT

PEDOT:DS Synthesis

Chemical synthesis was performed according to a published method⁵. Briefly 1 mmol EDOT monomer (Amsheng Chemical Co.) in Milli-Q H₂O (resistivity 18.2 M Ω cm) was combined with varying quantities of dextran sulphate (sodium salt, Sigma, MW 500,000), 1.2 mmol ammonium persulphate (Ajax Finechem) and catalytic quantities of iron(III) sulphate heptahydrate (Ajax Finechem) in a screw-top Schott bottle and mixed for up to 6 days with magnetic stirring. The resulting suspension was transferred to dialysis tubing (Sigma, MWCO 12,400 Da) and dialysed against Milli-Q H₂O for 3 days with daily changes of water.

For electrochemical synthesis, a 50 mL solution of Milli-Q H₂O (18.2 M Ω cm) containing 72 mg (0.01M) EDOT and 145 mg dextran sulphate was formed with the aid of 5 minutes of bath sonication. The 3-electrode electrochemical cell that was comprised of an Ag/AgCl reference electrode, platinum mesh counter electrode and an indium tin oxide coated (ITO) glass working electrode. Prior to use, the ITO glass was cleaned by 15 minute sonication in acetone followed by rinsing in Milli-Q H₂O, air drying and 5 minute plasma treatment (30W in air at 1100 mTorr). PEDOT:DS was synthesised by cyclic voltammetry (CV) using an EDAC potentiostat to apply 50 cycles between -0.4V – 1.2V at a rate of 50 mV/sec. CV current was observed to rise with cycle number in an approximately linear correlation (Figure 1A) and films of deep blue colouration were visibly deposited (Figure 1B). PEDOT:DS films were rinsed with Milli-Q H₂O and air dried prior to use.

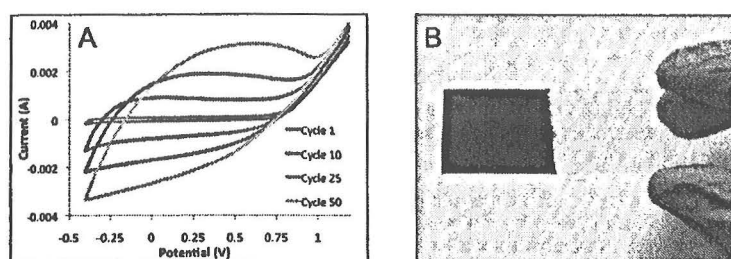


Figure 1. - A: selected cycles from the 50 cycle CV growth of PEDOT:DS. B: a fully-formed PEDOT:DS film on ITO glass.

UV-Vis

Absorbance profiles of PEDOT:DS films were determined using a Shimadzu UV-1800 spectrophotometer, scanning wavelengths between 350 and 1100 nm.

Post-polymerisation CV

Post-polymerisation CVs were obtained on electropolymerised PEDOT:DS as well as films of chemically polymerised PEDOT:DS that had been drop cast onto ITO glass at 70°C after the completion of dialysis purification. A 3-electrode cell was formed with the PEDOT:DS/ITO working electrode, platinum mesh counter electrode and an EDAQ ET072 leakless Ag/AgCl reference electrode, immersed in 0.1M tetrabutylammonium perchlorate (TBAP) in acetonitrile. Ten cycle CV measurements were conducted between -0.5 V and +1 V at a rate of 50 mV/sec.

Zetasizing/Potential

The size and stability of chemically synthesised PEDOT:DS nanoparticles was determined for 0.2 mg/mL suspensions of PEDOT:DS in Milli-Q water (18.2 MΩ cm). Sodium chloride was then added to yield final concentrations of 0, 10 and 100 mM NaCl. Resulting suspensions were analysed for hydrodynamic particle size and zeta potential using a Malvern Instruments Zeta Sizer following manufacturer protocols.

Wet State Conductivity

The conductivity and impedance behaviour of suspensions of chemically synthesised PEDOT was tested using a high surface area impedance device previously described by Warren *et al.*⁸. Briefly, two reticulated vitreous carbon (RVC) electrodes of varying thickness were placed at either side of a 1 cm wide channel such that the separation of the RVC electrodes varied from 0.5–2.5 cm. The channels were then filled with PEDOT suspensions and probed using an oscillating electrical potential of ± 15 mV with frequencies ranging from 1 - 100 000 Hz. By plotting resistance against channel length, the contact resistance and bulk solution resistance were independently determined.

SEM

High magnification (5000x) images of PEDOT:DS surfaces were obtained using a JEOL JSM7500FA Field Emission Gun Scanning Electron Microscope (FEGSEM) at 5 kV. Secondary electron images were collected via a semi in-lens detector at a working distance (WD) of 7 mm.

Cell Studies

Cell studies were performed on CV polymerised PEDOT:DS films on ITO glass, as well as ITO glass controls. Four well chamber slides (LabTek, Thermo Fisher Scientific) were removed from their bases and reattached to PEDOT:DS-coated ITO glass using a silicon sealant. After curing, wells were sterilised using 70% ethanol, dried and soaked in sterile Dulbecco's Modified Eagle's Medium (DMEM) prior to use. The rat fibroblast cell line, L929, was seeded at 20 000 cells/cm and grown over 96 hours in DMEM/F12 and 5% fetal bovine serum (FBS). Cells were imaged daily using light microscopy, with media changes every 2nd day.

DISCUSSION

In agreement with previous reports, both chemical and electrochemical synthesis were observed to reliably form PEDOT:DS, which presented as deep blue products in both suspension phase and solid films. UV-Vis spectra (Figure 2A) and post-growth cyclic voltammograms (Figure 2B) performed on electrochemically polymerised films and those cast from dialysed suspensions of chemically polymerised PEDOT:DS show similar responses. There was a significant difference in the time demands of the chemical process and subsequent purification (>1 week) compared with preparation time for CV polymerised films (~1 h), however electrochemical synthesis is reliant on the presence of a conductive substrate (in this case ITO glass) that limits the scalability of production and options for further processing. By contrast, the work of Harman *et al.* demonstrates the versatility of chemical synthesis for subsequent processing by spray coating, laser etching, ink-jet printing and extrusion printing of inks formed from the material⁵.

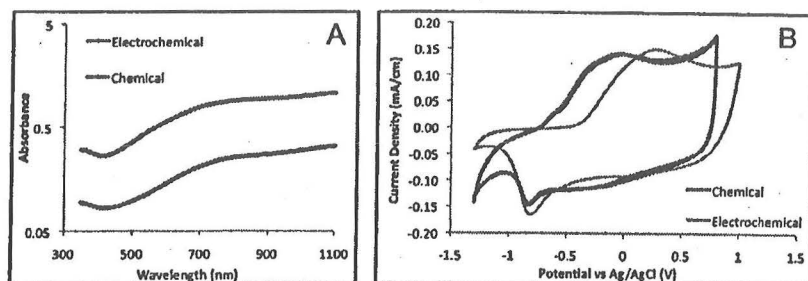


Figure 2. - Comparisons of electrochemically (blue) and chemically (red) polymerised PEDOT:DS films on ITO glass in terms of A: UV-Vis profiles and B: Cyclic voltammograms sweeping potentials of -1.3 – 1 V vs Ag/AgCl in 0.1 M TBAP/acetonitrile.

As with other organic conducting polymers, chemical polymerisation of PEDOT:DS forms a suspension of discrete nanoparticles. Conductivity measurements conducted directly on PEDOT:DS suspensions revealed conductivities of the order of 6-8 mScm⁻¹ (Table I), several orders of magnitude lower than those reported for cast films (up to 19.6 Scm⁻¹). It is likely that this discrepancy is due to a lack of direct connection between neighbouring PEDOT:DS nanoparticles in suspension, resulting in a prohibitively high charge transfer resistance for electronic conduction. In other systems, this has been overcome by increasing the concentration of conductive elements until the percolation threshold is reached, whereby sufficient interconnections are established between conductive elements to form a continuous conductive pathway⁸. This approach is unlikely to be suitable for PEDOT:DS in biological applications however, as the stability of PEDOT:DS nanoparticles is compromised by the addition of salt, manifesting as a reduction in surface potential and hydrodynamic volume at millimolar NaCl concentrations (Table II) and the formation of aggregates visible in cell culture microscopy. Effectively, this places an upper limit on the achievable concentrations of dispersed PEDOT:DS under physiological conditions that is lower than the percolation threshold required for bulk phase conductivity. Because of this effect, we believe PEDOT:DS alone will not find application as a bulk conductive element in biological systems, however surface coating or blending approaches remain viable.

Table I – The conductivity determined for 0.2 mg/mL suspensions of chemically polymerised PEDOT:DS doped with DS at ratios between 1:1 and 1:3. Saline is included for comparison.

System	Conductivity (mS/cm)
PEDOT:DS 1:1	6.5 ± 1.1
PEDOT:DS 1:2	6.9 ± 1.7
PEDOT:DS 1:3	7.6 ± 2.9
Saline (154mM NaCl)	13.8 ± 2.4

Table II – Zeta size and potential data for dispersed suspensions of chemically polymerised PEDOT:DS (1:2) with increasing concentrations of NaCl.

System	Particle Size (nm)	Zeta potential
PEDOT:DS (1:2)	593 ± 58	-63.2 ± 1.7
PEDOT:DS (1:2) + 10mM NaCl	452 ± 37	-48.1 ± 3.0
PEDOT:DS (1:2) + 100mM NaCl	363 ± 7	-39.9 ± 2.0

The nanoparticulate nature of chemically synthesised PEDOT:DS also impacts on the topography of cast films. SEM images taken on drop-cast films (Figure 3A) show substantially greater roughness than their electropolymerised counterparts (Figure 3B). This difference is significant as the amount of available surface area impacts upon a surface's electrochemistry^{9, 10}, and surface topography is also known to significantly impact on the behaviour of cells^{11, 12}. Altering the conditions of chemical synthesis was shown by Harman *et al.* to impact the sizes of resulting PEDOT:DS particles⁵. Leveraging this effect may allow for the tailoring of surface roughness, and is a significant advantage of the chemical synthesis technique. However in the present study, films of chemically polymerised PEDOT:DS drop-cast on glass surfaces were observed to delaminate and disintegrate with prolonged exposure to aqueous solvents. The

highly hydrophilic nature of dextran sulphate combined with the roughness of chemically synthesised PEDOT:DS films were considered the likely causes for this instability. The application of chemically synthesised PEDOT:DS as surfaces for cell growth and/or electrical stimulation would be particularly challenging due to the long immersion times, elevated temperatures and frequent washing procedures typical of cell culture. PEDOT:DS surfaces for cell application therefore will likely be better served by electrochemical polymerisation.

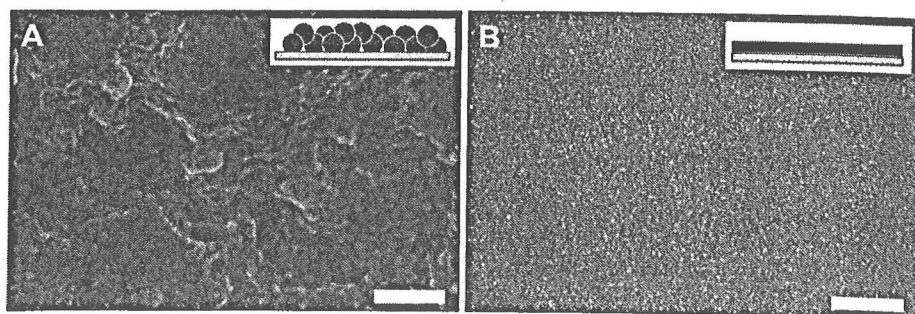


Figure 3 – SEM images of A: Drop-cast chemically polymerised PEDOT:DS and B: PEDOT:DS electropolymerised on ITO. Insert: Schematic diagrams of PEDOT:DS morphology. Scale bar represents 100nm

To examine the impact of the synthesis method on cell behaviour, the L929 fibroblast cell line was seeded onto PEDOT:DS produced both chemically and electrochemically. Films of electrochemically synthesised PEDOT:DS were not visibly impacted by immersion in culture media over the 4-day study. Cells grown on these materials (Figure 4 B1-B4) were observed to attach and spread, exhibiting morphologies typical of cultured L929 cells and appeared to be minimally impacted by the presence of the PEDOT film. The stability and cytocompatibility of these electropolymerised films make them good candidates for the direct electrical stimulation of cells *in vitro*, and they may also find application as a coating for cell communication *in vivo* as has been previously suggested for PEDOT:PSS and other conductive polymers². Due to the instability of chemically polymerised PEDOT:DS films, the cytocompatibility was tested by exposing cells to suspensions of 0.2% (w/v) PEDOT:DS in culture media (Figure 4 A1-A4). Even at these low concentrations some aggregation of PEDOT:DS was observed. In line with previously reported zeta potential data, it is likely that the PEDOT:DS particles were destabilised by the exposure to high ionic strength of cell culture media. Moreover, because dispersed PEDOT:DS particles do not substantively elevate solution conductivities, chemically polymerised PEDOT:DS appears unsuitable for the direct electrical stimulation of cultured cells. Notably the aggregates of PEDOT:DS were visibly localised to the L929 cells, suggesting that the cells may be either internalising the dispersed PEDOT:DS particles, or adhering them to the cell wall. In spite of this proximity, the viability of L929 cells was not detrimentally affected by the presence of the aggregates over the 4-day period. Previous work demonstrating the drug loading capacity of PEDOT^{13,14} suggests the possibility of co-doping PEDOT with both drugs and DS. Such a co-doped species may lead to the delivery of drugs to, or across, the cell membrane, an application of PEDOT:DS which warrants further investigation.

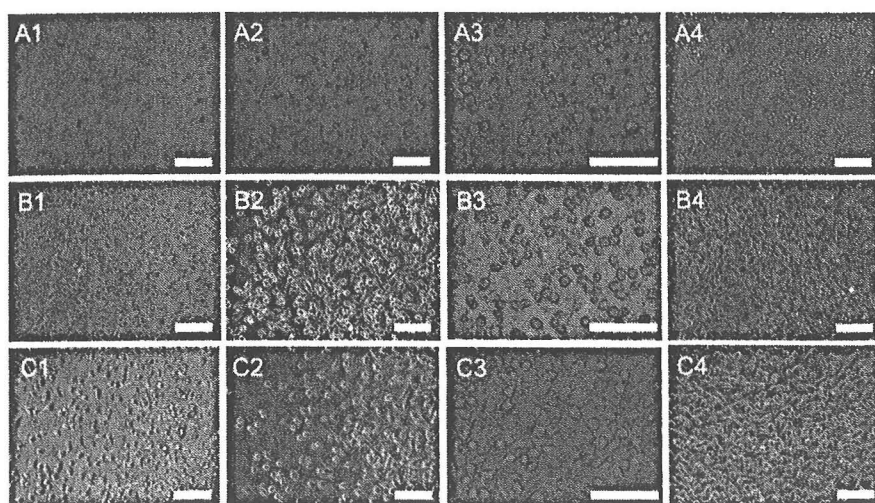


Figure 4 – Four day culture of L929 cells in the presence of PEDOT:DS. Numbers represent days in culture with either chemically synthesised PEDOT:DS (A1-A4), electrochemically grown PEDOT:DS films on ITO (B1-B4, reproduced from Harman *et al.*³) or control cells on uncoated ITO glass (C1-C4). Scale bars represent 100 μm .

CONCLUSIONS

Dextran sulphate (DS) is a polysaccharide recently reported as a biodopant for the organic conductor PEDOT⁵⁻⁷. Here, two synthesis modes of PEDOT:DS, chemical and electrochemical, have been directly compared. Chemical synthesis of PEDOT:DS yielded a suspension of discrete PEDOT:DS nanoparticles with good inherent conductivity and previous work has demonstrated the versatile processability of this material⁵. Bulk suspensions of PEDOT:DS however, possess minimal conductivity, attributed to the lack of interconnecting conductive pathways within PEDOT:DS suspensions at achievable polymer concentrations. Realising the material's good fundamental conductivity requires casting and drying films to re-establish these pathways. SEM imaging of cast films of chemically polymerised PEDOT:DS exhibited substantial surface roughness attributable to the particulate nature of the dispersion. Whilst this surface roughness may be beneficial in energy applications, cell culture is hampered by the instability of these films when exposed to cell culture media. Notably, dispersed chemically polymerised PEDOT:DS appears to localise at proliferating L929 cells, suggesting a possible application for the material as a drug delivery vehicle. Cell communication and cell stimulation applications, being reliant on continuous conductive pathways for the delivery of electrical signals to the cell, are problematic with chemically synthesised PEDOT:DS. Electrochemical synthesis however, yielded films that were stable under cell culture conditions during 4-day studies with L929 cells. SEM imaging revealed substantially reduced roughness. Cells grown on electrochemically polymerised PEDOT:DS films were morphologically similar

to these cells under typical cell culture conditions. For these reasons, electrochemically synthesised PEDOT:DS is considered better suited for cell culture and electrical stimulation applications. Overall this work supports the use of dextran sulphate as a novel biopolymer for PEDOT, with synthesis method of considerable importance in targeting this application.

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