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Printed biotin-functionalised polythiophene films as biorecognition layers in the development of paper-based biosensors

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ABSTRACT

The integration of flexible electronic sensors in clinical diagnostics is visioned to significantly reduce the cost of many diagnostic tests and ultimately make healthcare more accessible. This study concentrates on the characterisation of inkjet-printed bio-functionalised polythiophene films on paper-based ultrathin gold film (UTGF) electrodes and their possible application as biorecognition layers. Physicochemical surface properties (topography, chemistry, and wetting) and electrochemical characteristics of water-soluble regioirregular tetraethylene-glycol polythiophene (TEGPT) and biotin-functionalised TEGPT (b-TEGPT) films were examined and compared. In addition, their specificity towards streptavidin protein was tested. The results show that stable supramolecular biorecognition layers of insulating b-TEGPT and streptavidin were successfully fabricated on a paper-based UTGF by inkjet-printing. Good adhesion of thiophene to UTGF can be attributed to covalent linkage between sulphur and gold, whereas the stability of the streptavidin layer is due to the high affinity between biotin and streptavidin. The device introduced can be utilised in the development of biosensors for clinically relevant analytes e.g. for detecting complementary DNA oligomers or antibody–antigen complexes.

1. Introduction

Presently, great academic and industrial level interest is seen in the development of electronic sensors fabricated on a flexible paper substrate (paper electronics), to be applied in the field of clinical diagnostics [1]. This is driven by the vision that the use of paper electronics – lightweight, thin and low-cost devices – could significantly contribute to the development of easy-to-use point-of-care diagnostic solutions and ultimately make health care more accessible. In addition, the adaptation of emerging technology fields such as printing to biosensor development may greatly improve the precise control and repeatability of biomaterial processing [2].

The most important requirement for obtaining a sensitive biosensor surface is that the binding activity and specificity of the immobilised biorecognition components are preserved towards analyte. A biorecognition layer should also possess a homogeneous structure and a low degree of nonspecific binding, and be stable for long-term use. Various methods for coupling biomolecules on solid surfaces are available, including direct physisorption, covalent binding directly to the substrate or via a mediator such as self-assembly monolayers (SAMs) or biofunctionalised polymers, or affinity coupling via e.g. biotin–avidin linkage [3–5]. Recently, the possibility of using paper-supported gold electrodes in diagnostic applications has been demonstrated, showing that various biorecognition surfaces can be constructed and clinically relevant analytes can be detected by electrochemical techniques such as electrochemical impedance spectroscopy (EIS) [6–8].

Biofunctionalised polymeric materials are considered as suitable materials in the development of printable biosensors, due to their relative good processability and high degree of intergratability to signal transducers such as transistors and chemoresistors [9]. Among these materials, polythiophene and its derivatives have emerged as one of the most promising materials due to the ease of synthesis, environmental stability and the possibility to modify it with different chemical groups [10–12]. Substituted polythiophenes can be tailored for various applications by designing the side groups to give the polymer different functions.

The aim of this study was to examine the possibility of using biotin-functionalised polythiophene as a base material in a biorecognition layer of a printed paper-based biosensor. Fig. 1

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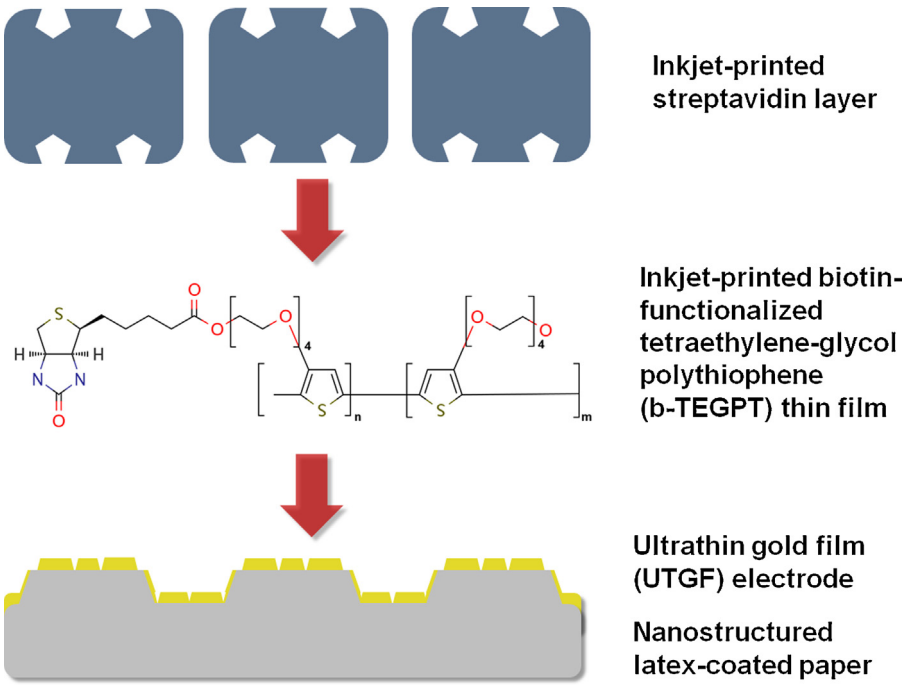

Fig. 1. A schematic presentation of different components and fabrication steps of the supramolecular bio-recognition layer devised on the nanostructured latex-coated paper-supported UTGF electrode, including the chemical structure of b-TEGPT ($n \sim 9$ and $m \sim 1$).

shows schematically the intended structure. The design consists of ultrathin gold film (UTGF) electrode contacts evaporated on nanostructured latex-coated paper on which the biotin-functionalised polythiophene is inkjet-printed. The biotin-functionalised polymer film offers a highly bioadhesive layer for inkjet-printed streptavidin proteins through the extremely high biotin–streptavidin affinity (dissociation constant of 10^{-14} mol L^{-1}) [13]. Physicochemical properties and impedimetric characteristics of the inkjet-printed biotin-functionalised polythiophene films and subsequent streptavidin layers were studied. In addition, the specificity of the polymer film towards the streptavidin protein was inspected. These printed supramolecular constructs could in the future be used as anchoring layers for impedimetric detection of e.g. biotin-functionalised antibodies or DNA oligomers.

2. Materials and methods

2.1. Paper substrate

A multi-layer curtain-coated paper with excellent barrier properties against water and solvent penetration was used as the base paper [14]. The base paper was further coated with an aqueous dispersion of a latex blend containing carboxylated styrene butadiene acrylonitrile (ABS) copolymer (T_g = 8–10 °C, DL920, DOW Chemicals) and modified polystyrene (PS) copolymer (T_g < 90 °C, DPP3710, DOW Chemicals) with the final ABS:PS weight ratio of 3:2. The nanostructured surface texture was created by irradiating the latex-coated paper with a short-wavelength infrared heater (IRT systems, Hedson Technologies AB, Sweden) for 60 s (Appendix Fig. A.1). Details on the fabrication of nanostructured latex-coated papers have been described elsewhere [15–18].

2.2. Preparation of the ultrathin gold film electrodes

Physical vapour deposition was used to fabricate ultrathin gold films (UTGFs) on the nanostructured latex-coated paper by using a shadow mask for electrode patterning (Appendix Fig. A.1). The evaporation was done under high vacuum (10^{-6} mbar) during two separate runs, with the evaporation rate set to 12 nm min^{-1}. A deposition monitor (XTM/2, Inficon) was used for gravimetric determination of the amount of evaporated gold on the latex surface. The nanostructured latex coating has been shown to offer excellent adhering characteristics towards UTGF without a need of additional adhering interlayers [19]. UTGFs with nominal thickness of 20 nm had an average grain height of 6.2 ± 0.3 nm and the resistivity of 2.6×10^{-6} Ω cm [19]. The combined area of the working and the counter electrodes was 0.2 cm^2.

2.3. Biotin-functionalised polythiophene

The water-soluble regioirregular tetraethylene-glycol polythiophene (TEGPT) and biotin-functionalised TEGPT (b-TEGPT) were synthesised as described in literature [20–25] and the details are given in Appendix Fig. A.2. The ratio of non-biotinylated (n) and biotinylated (m) units in b-TEGPT was about $n \sim 9$:$m \sim 1$ (Fig. 1).

2.4. Inkjet printing

The TEGPT and b-TEGPT inks were formulated by mixing the aqueous reddish-black polymer solutions (concentration: 5.9 mg/ml) and ethylene glycol (EG) in ratio of 70:30 vol% (Appendix Fig. A.3A). The physicochemical properties of the ink (at 25 °C) were as follows: surface tension 63 mN/m, viscosity 4.3 cP and density 1.0135 kg/L [26]. These values yielded a Z number of 8.6 for the ink, predicting good inkjet jettability [27,28].

The b-TEGPT and TEGPT inks were inkjet-printed on the active area (0.2 cm^2) of the UTGF electrode using a Dimatix Materials Printer (DMP-2831, FUJIFILM Dimatix, Inc.) and a cartridge with 10 pL nominal drop volume. The jetting parameters were as follows: drop spacing 30 μm (1156 drops/mm^2), firing voltage 15 V and firing frequency 2 kHz. The ink cartridge temperature was set to 30 °C. This resulted in a drop diameter of 28 ± 2 μm (Appendix Fig. A.3B). In addition, different surface treatments and heating setups were tested to obtain the most homogenous film quality for the