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A contemporary approach for design and characterization of fiber-optic-cortisol sensor tailoring LMR and ZnO/PPY molecularly imprinted film

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ABSTRACT

A fiber optic salivary cortisol sensor using a contemporary approach of lossy mode resonance and molecular imprinting of nanocomposites of zinc oxide (ZnO) and polypyrrole (PPY) is structured and depicted for the concentration range of 0–10^{-6} g/ml of cortisol prepared in artificial saliva. Components of polymer preparation and the nanocomposite of polymer with ZnO are optimized for realizing the molecular imprinted layer of the sensor. Nanocomposite having 20% of ZnO in PPY is found to give highest sensitivity of the sensor. The sensor reports the best limit of detection ever reported with better stability, repeatability and response time. Lossy mode resonance based salivary cortisol sensor using nanocomposite molecular imprinted layer reported first time boosts the specificity of the sensor. The implementation of sensor over optical fiber adds up other advantages such as real time and online monitoring along with remote sensing abilities which makes the sensor usable for nonintrusive clinical applications.

1. Introduction

Cortisol, also named as hydrocortisone, is a steroid hormone released by the chain operation of hypothalamus, pituitary and adrenal glands in response to a circumstantial trigger to human body. The HPA (hypothalamus-pituitary-adrenal) axis releases corticotrophin releasing hormone to the pituitary gland which in turn stimulates its cells to generate adrenocorticotrophic hormone. In blood, the hormone released from the pituitary cells transits to adrenal gland, where the zona fasciculate in the adrenal cortex releases cortisol in response to it. Cortisol plays an important lead in maintaining the human homeostasis through the negative feedback HPA system. Up to an extent, certain changes in the human homeostasis are beneficial to the body by activating the psychological-physical betterment and movement. The extended exposure to such triggers results in an abnormal increase in cortisol secretion which immensely affects the human immune, skeletal, cardiovascular, endocrine, and renal system as well as the glucose, blood pressure and carbohydrate metabolic levels. The decrement and the increment in cortisol level affect the human system by causing Cushing's diseases and Addison's diseases respectively, which show the importance of its range in human body. The substantial variation in its range is due to human exposure to psychological/environmental/emotional stress and hence cortisol has been considered as the major stress hormone (Kaushik et al., 2014).

Analysis of cortisol level in human body can be performed from samples of blood, sweat, hair, urine, interstitial fluids and saliva (Singh et al., 2014). Since the cortisol levels in saliva holds a strong analogous to that of the blood, the most opted sample for analysis is saliva. Around 90% of free cortisol is present in human saliva and hence it almost equivalents the range of free cortisol level in human blood (Tlili et al., 2011). The cortisol level in saliva varies in day and night times as well as with individuals, especially for those with variations in cardiac rhythm. The range will differ highly for even healthy and weak individuals, with and without body exercise. Hence a wide range should be covered by the stress biomarker for cortisol quantification (Tahara et al., 2014). Several clinical application techniques have been in use for salivary cortisol detection such as ELISA (enzyme-linked immunosorbent assay), fluorescent polarization, laser induced fluorescence, QCM (quartz crystal microbalance), electrochemical immunoassay, RIA (radio immunoassay) and electrochemical impedance spectroscopy. These methods suffers one or more of the disadvantages such as expensive, complicated system which does not support online monitoring and remote sensing, portability issue, time consuming analysis, tedious procedure having multiple steps, continuous man power requirement, poor selectivity, requirement

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of hardware that does not make the system miniaturized and high power consumption (Tlili et al., 2011; Tahara et al., 2014; Singh et al., 2014).

Recently, a novel approach of tailoring lossy mode resonance (LMR) and molecular imprinting polymer (MIP) techniques has been reported by us for salivary cortisol sensing which overcomes all the disadvantages discussed above (Gupta et al., 2016). Moreover, the use of optical fiber facilitates advantages of real time analysis along with multi-anlayte detection (Tabassum and Gupta, 2016). LMR can be generated by replacing the cladding of a highly multimode optical fiber with the semiconducting metal oxide (SMO) layer which results in the coupling of the evanescent wave at the fiber core-SMO interface with the guided modes of the SMOs. These guided modes are lossy in nature due to low value of the imaginary part of the dielectric constant of the SMOs in comparison to its positive real part and the imaginary part of the dielectric constant of the medium surrounding the SMO (Villar et al., 2010, 2012). Zinc oxide (ZnO) has been mostly opted to implement the LMR technique due to its biocompatibility and other supporting properties such as high isoelectric point (9.5), native point defects, non-toxicity, stability, inertness, non-specific adsorption, enhancement in enzyme activity, piezoelectric, optical properties and reduction in product inhibition which make ZnO a better matrix, carrier and material for multiple applications (Usha et al., 2015a, 2015b; Villar et al., 2010, Janotti and Walle, 2009, Usha et al., 2016).

Molecular imprinting is a technique for the highly selective detection of analyte to be quantized by tailor made memory/recognition encampments (Verma and Gupta, 2013; Shrivastav et al., 2016). The interaction between the functional materials (monomer) and the template molecule supports the adsorption of specific analyte on the memory encampments by their arrangement, direction, shape and size (Opik et al., 2009). Amongst the different polymer materials, conducting polymers exhibit unique electrical, chemical and optical properties in addition to inertness, biocompatibility, ease of preparation and polymerization, ability to transit between the conducting and insulating state by oxidizing and reducing properties, stability, reversibility and superior conductivity (Opik et al., 2009; Chougule et al., 2011; Batool et al., 2012). The conjugated double bond supports the metal like behavior in polypyrrole (PPY), and can be polymerized from the monomer pyrrole (PY) in either organic or aqueous solutions to support easy imprint of bio-chemical-molecules for sensing purposes (Schweiger et al., 2015; Chougule et al., 2011; Batool et al., 2012). A high performance hybrid material of PPY by polymerizing PY in the presence of ZnO has been reported (Batool et al., 2012). ZnO has already proved its role as a material in LMR sensors (Usha et al., 2015a, 2015b), sensing material (Janotti and Walle, 2009) and matrix material in sensor probe for enzyme/protein loading (Usha et al., 2016), with its high refractive index and isolelectric point, thermal stability, high electron mobility etc.

In this study, a LMR based fiber optic non-invasive stress biomarker for sensing of salivary cortisol is reported. The sensor is fabricated by coating a ZnO film over unclad core of the fiber followed by the coating of a nanocomposite film of the optimized polymer. A cortisol concentration range of 10^{-12}–10^{-6} g/ml is chosen for the sensor to cover the changing levels of salivary cortisol due to various environmental and bodily conditions. The primeval LMR-nanocomposite based stress biosensor is characterized using artificially prepared saliva and the results obtained are compared with the existing sensors.

2. Experimental

2.1. Preparation of polymer and artificial saliva samples

The molecular imprinting technique for the conducting polymer like PPY involves the usage of monomer, oxidizing agent, solvent and template/analyte molecule. In our previous preliminary study, PPY was prepared using the reagents – PY (monomer), ethanol (solvent) and ferric ferrocyanide (oxidizing agent) (Gupta et al., 2016). For the preparation of this, two separate solutions (3.4 ml of PY in 25 ml ethanol and 0.1 M of ferric ferrocyanide in 25 ml of ethanol) were prepared, mixed at room temperature and was left for 24 h for polymerization. For optimization purpose, PPY was prepared using two different methods also. Following the method given by Chougule et al. (2011), a chemical oxidative procedure was used for the polymerization of PY in ammonium persulphate (APS) in a ratio of 1:1. For this 0.174 ml of PY was sonicated in 25 ml of ethanol and 0.57 g of APS was sonicated in 25 ml of ethanol separately. The two solutions were homogenized together at room temperature for 4 h for polymerization. Since ferric chloride has also been reported as an oxidizing agent for the preparation of PPY, another PPY was prepared by homogenizing 3.4 ml of PY dissolved in 25 ml of ethanol and 2.7 g of ferric chloride sonicated in 25 ml of ethanol for 4 h at room temperature (Tan and Ghandi, 2013). There is a slight difference in the preparation of PPY using ferric chloride as oxidizing agent in the present study and used by Tan and Ghandi (2013). They have used D_2O as solvent while in the present study ethanol has been used as solvent. The change was made to enhance the properties of MIP layer in supporting sensing. It may be noted that the polymerization of PY to PPY, above, has been carried out chemically using three different oxidizing agents-ferric ferrocyanide, ammonium persulphate and ferric chloride. The polymerization process occurs fast and steadily in the presence of these oxidizing agents (Tan and Ghandi, 2013). For polymerization of PY to PPY, on an average 4 h are required (Chougule et al., 2011). However, the mixture was kept for 24 h for proper binding within the polymer, after which, the template molecule-cortisol of 1.5 mM was added to the PPY and ultra-sonicated for 1 h. The polymer-template mixture was then left for 24 h for proper binding between template and polymer otherwise the removal procedure of template from the polymer, to create the molecular imprinted structure, can deform the imprints in the polymeric layer (Shrivastav et al., 2016). The performance of the sensors fabricated using coating of PPY prepared employing three different oxidizing agents was tested and the one giving the best results out of three oxidizing agents was used for further studies.

To prepare the ZnO/PPY nanocomposite with varying weight percentage of ZnO/ PPY in the composite, the measured quantity of ZnO was dispersed in a solution having 0.57 g of APS mixed in 25 ml of ethanol. The solution with ZnO was mixed in a magnetic stirrer properly. To this mixture, 25 ml of ethanol with 0.174 ml of PY was added and kept on magnetic stirrer for 4 h to obtain the nanocomposite. The weight percentage of ZnO/PPY was varied as 2.5%, 5%, 10%, 20% and 30% (Batool et al., 2012). The approach used for the preparation of nanocomposite in the present study is similar to that used by Batool et al. (2012). The difference is in the chemicals used for the preparation of PPY. For example, we have used APS as the oxidizing agent and ethanol as the solvent while they have used ferric chloride hexahydrate and distilled water as the oxidizing agent and solvent respectively. The need of change in chemicals in our study arose due to the requirement of incorporating molecular imprinting technique for the sensing of specific analyte.

For the preparation of artificial saliva, 0.4 g of sodium chloride, 0.6 g of di-sodium hydrogen phosphate, 4 g of urea, 0.6 g of