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Colorimetric sensor based on bubble wrap and camera phone for glucose determination

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ABSTRACT

Glucose is a typical marker of diabetes mellitus but it corresponds also with metabolic syndrome, immune disorders and some types of poisoning. Hence fast, low cost and simple method for its determination is required. Bubble wrap, cheap and available material, was used for immobilization of glucose oxidase (GOx) and peroxidase (POx), the enzymes necessary for colorimetric determination of glucose. Method is based on reaction of enzymes (immobilized in sol-gel membrane inside the bubbles) with substrates glucose and o-phenylenediamine dihydrochloride (o-PD) providing intensive coloration. The color change can be easily tracked by phone integrated camera. Color intensity expressed in red-green-blue (RGB) color model was used for displaying of photos and for gaining numeric data representing concentrations of glucose. The assay exerted good correlation of color intensity with the concentration of glucose, adequately low limit of detection (750 mmol/l) for glucose blood assay, no influence of interferents or matrix substances and by sufficient long term stability of sol-gel membrane. The sensor was found as low-cost simple way to analyze glucose blood levels with promising prospects in the field of portable devices.

Introduction

Glucose is very important metabolic substance in physiological functioning of human body. It is an essential substrate for human brain, which utilized about quarter of glucose circulating in blood stream. It is also necessary for muscles including heart and bone muscles and for other tissues (Sabzghabaee et al., 2011; Sonneville et al., 2015). Abnormal glucose level is crucial diagnostic marker. It is helpful during revealing and necessary in therapy of pathologies like abnormal glucose metabolism (diabetes mellitus, metabolic syndrome). It is also helpful in therapy of pathological immune response, use of glucose level affecting drugs (counter regulatory hormons) or poisoning (sarin), and glucose level may represent a problem in hospitalized patients (Sabzghabaee et al., 2011; Sonneville et al., 2015; Pohanka et al., 2012; Brutsaert et al., 2014). Fast and accurate method may reveal disease in time and prevent development of lethal conditions. It is noteworthy that American Diabetes Association recommended accuracy 15% for glucose meters used in home care glucose assay but most of the devices do not fit this condition (Tonyushkina and Nichols, 2009). Determination of glucose from whole blood, where glucose is highly instable and determination of glucose from capillary blood where glucose level is about 20% higher than vein blood glucose level, is another problem (Tonyushkina and Nichols, 2009). So we can say that currently available methods for home care glucose assay are not adequate for this time.

The well-known principle of glucose biosensor was invented by Clark and Lyons in 1962, when researches needed to assess glucose levels during their exploration of artificial blood (Clark and Lyons, 1962). Our measurement is based on the same reaction of glucose with enzyme GOx where gluconolactone is a product and hydrogen peroxide is a co-product. Hydrogen peroxide is used in following reaction, where it is utilized by enzyme POx for transformation of substrate o-PD into color product. The color change is measurable by spectrophotometer (Golden and Sapir, 2012; Turner, 2013; Martinkova and Pohanka, 2015). Currently, phone camera is accessible technique for regular population so the idea of application of camera phone into detection of color change arose (Lu et al., 2009; Chuen et al., 2014).

Sol gel is a type of nanomaterial, which is appropriate for immobilization of biomolecules like enzymes. It is prepared by

Abbreviations: GOx, glucose oxidase; oPD, o-phenylenediamine dihydrochloride; POx, peroxidase;; RGB, red/green/blue color model.

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hydrolysis and condensation of alcoxide in period of time while see-through glass-like membrane is forming. Immobilization of large amount of biomolecules, physical and chemical stability, simplicity and flexibility of prepared membrane are the significant properties of the immobilization technique (Singh et al., 2007). It is also well-liked for its homogenous, porous and dense network structure appropriate for particle interaction (Akhtar et al., 2016; Islam et al., 2016).

Our intention was to prepare simple but precise glucose sensor using available and low-cost materials and techniques. Bubble wrap and phone camera do fit this idea (Kumar et al., 2000; Bwambok et al., 2014). Aim of this work was to integrate bubble wrap to bioassay as a container of enzymes and sol-gel was intended as immobilization membrane of them. To reach affordable, accessible and portable device, we planned to use phone camera as a detector of reaction color change. It was also in our mind to compare our assay with reference one and evaluate contribution of novel method. It is expected that the assay will be readily to practical use with minimal costs but with retained sensitivity to glucose.

Materials and methods

Chemicals

All chemicals apart from plasma samples were obtained from commercial sources in at least analytical grade. They were used without further treatment apart of solution making. Glucose, sucrose (4 mmol/l), sorbitol (4 mmol/l), deoxyribose (4 mmol/l), maltose (4 mmol/l), fructose (4 mmol/l), reduced glutathione (4.5 μmol/l), trolox (50 μg/ml), urea (20 mmol/l), bovine serum albumin (80 mg/ml), GOx from *Aspergillus niger*, type VII (4 mg/ml), POx from horseradish type VI (0.5 mg/ml), o-PD, tetraethyl orthosilicate, triton X-100 (10% solution) were purchased from Sigma Aldrich (Saint Louis, Missouri, USA). Ascorbic acid (20 μg/ml), hydrochloric acid (35%), sodium acetate trihydrate and acetic acid were bought from Penta (Prague, Czech Republic). Acetate buffered saline (pH 5.0) for preparation of sol-gel was made from 0.1 mol/l acetic acid and 0.1 mol/l sodium acetate mixed together in 1:1 ratio. Sodium acetate buffer (50 mmol/l, pH 5.5) was prepared from sodium acetate trihydrate. Solution of o-PD was prepared by mixing of 3.5 mg of o-PD powder with 50 ml of sodium acetate buffer. Demineralized water was prepared by reverse osmosis process using device Aqua Osmotic 2 (Aqua Osmotic, Tisnov, Czech Republic). In total 15 blood samples were obtained post mortem from mice after sacrificing of the animal in Faculty of Military Health Sciences, University of Defense (Hradec Kralove, Czech Republic) vivarium facility. Animal manipulation and sacrificing was permitted and supervised by ethical committee in the Faculty of Military Health Sciences. Blood was taken to heparinized tubes and plasma was received by centrifugation at 3000 RPM for 10 min.

Apparatus

Images were taken by camera phone Sony Xperia MT27i (Tokyo, Japan) and samples were measured in pentaplicate under standard ambient temperature and pressure conditions. Sol-gel was mixed by the heating magnetic stirrer AREX from Velp Scientifica (Bohemia, New York, USA) and solution for its preparation was preheated using thermostat incubator NB-203 from N-Biotek (Bucheon-si, South Korea). Buffers and other solutions were prepared using pH meter CyberScan pH 6000 from Eutech (Landsmeer, The Netherlands), semimicro balance CPA225D from Sartorius (Göttingen, Germany) and personal vortex V-1 plus from Biosan (Riga, Latvia).

Data processing

Color intensity of photos was gained by converting each image into numeric data using RGB image processing GIMP 2.8.14 (free and open source software). Measured data were processed using Origin 9.1 software (OriginLab Corporation, Northampton, Massachusetts, USA). Signal vs. noise equal to three criterion (S/N = 3) was used for limit of detection calculation.

Preparation of sol-gel membrane

Used sol-gel preparation technique was invented by Reddaiah and Madhusudana Reddy (Reddaiah and Madhusudana Reddy, 2014). Our procedures were slightly changed: all chemicals for sol-gel preparation were preheated in incubator up to 37 °C. Warmed tetraethyl orthosilicate, triton X-100, hydrochloric acid and water were mixing on the heating magnetic stirrer (40 °C) till the clear sol was created (approximately 2 h). Hot clear sol was added into acetate buffered saline solution with enzymes also warmed up to 37 °C. Mixture of enzymes GOx and POx were prepared in ration 1:8. 50 μl of prepared sol-gel was injected into bubbles of packing bubble wrap. Bubble wrap was put into incubator (37 °C) and sol-gel was solidified after 30 min of incubation.

Concentration curve

Bubble wrap (size of 35 bubbles—7 concentration points measured in pentaplicate) filled with solid sol-gel membrane with immobilized enzymes GOx and POx was used for measuring of concentration curve. Water and glucose in concentration range from 1.0 to 24 mmol/l was used as standard solutions. Standard solutions (0, 1.0, 2.0, 4.0, 8.0, 16 and 24 mmol/l) were mixed together with o-PD solution (glucose was diluted 5 times) and 120 μl of this mixture were injected into bubbles. Reaction was taken 10 min and then the photos of bubbles were taken from constant distance 20 cm in dark with flash in the phone switched on and automatic balance of white. Photos were uploaded into personal computer, where color intensity of three points of each bubble were analyzed using the RGB color model. Concentration curve of color intensities in each color channel was fitted.

Interferences

Interferents are substances with similar structure or containing the same reaction group as the measured substance. In case of glucose, sugars deoxyribose, sucrose, sorbitol, maltose and fructose were chosen as typical interferents in glucose assay. So the glucose, water or interferents in concentration 4 mmol/l were mixed with o-PD and diluted 5 times, injected into bubbles and color change were recorded by phone camera after 10 min. Data were gained the same way as was described above in chapter Concentration curve.

Matrix effect

Matrix effect is measured following way: substances present in biological samples (ascorbic acid, reduced glutathione, trolox as a water soluble derivative of vitamin E, urea and bovine serum albumin) in concentration twice higher than their physiological were mixed together with 8 mmol/l glucose in ration 1:1. Final concentration of glucose in the samples was 4 mmol/l and concentration of interferents replied their physiological concentration in human plasma. These solutions were measured the same way as concentration curve, so they were diluted 5 times with o-PD, injected into bubbles and after 10 min of reaction the photos

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