Flexible cotton-AuNP thread electrode for non-enzymatic sensor of uric acid in urine

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Abstract We report on the development of an electrochemical sensor platform based on modified cotton fibers for the non-enzymatic detection of uric acid (UA), an important biomarker for gout disease. To create the flexible electrode, a cotton thread was coated with carbon ink as a pre-conductive layer prior to direct electrodeposition of AuNPs. Then, differential pulse voltammetry (DPV) was used to evaluate the sensor performances, and a linear detection range between 10 µM and 5.0 mM of uric acid was obtained. The sensor has a detection limit of 0.12 µM, which is sufficient for use in the patients suffering from gout disease which uric acid is higher than 4.46 mM. Furthermore, we found that the detection sensitivity of the platform was not affected by the presence of other physiological compounds present in human urine. The described platform has the potential for integration in a diaper hence enabling rapid detection and screening for gout disease.

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**Introduction**

Non-invasive diagnosis allows for the direct detection of target biomarkers from body fluids without the common pain and infection risk associated with blood-based protocols (Li et al. 2019a; Michelena et al. 2019; Eftekhar et al. 2019; Doménech-Carbó et al. 2018). Various substrates have been used for non-invasive diagnosis including tattoos (Bandodkar et al. 2013; Jia et al. 2013), paper-based sensors (Anastasova et al. 2017), stretchable elastomers (Martin et al. 2017) and textile substrates (Liu and Lillehoj 2017). Textiles have become an attractive material owing to their flexibility, biocompatibility and high-water absorption (Caldara et al. 2016; Promphet et al. 2019; Ren et al. 2017). Among textile-based sensors, fabric platforms usually require of a high volume of biofluid sample, while yarns and threads require of low sample volumes and offer unique microfluidic properties (Ahmed et al. 2019; Reches et al. 2010; Oliveira et al. 2019; Liu et al. 2018, 2010; Polanský et al. 2017; Weng et al. 2019; Yokota et al. 2008b; Sugano et al. 2016; Akhlaghi et al. 2013; Ruecha et al. 2020; Magosso et al. 2012; Wenz and Liepold 2006; Wu et al. 2012).

Uric acid (UA), chemically designated as 2, 6, 8-trihydroxypurine, is a major product of the catabolism of purine nucleosides, adenosine and guanosine. The normal concentration of uric acid in human urine varies between 1.4–4.46 mM and values higher than 4.46 mM are used for gout disease diagnosis (Azmi et al. 2015; Liu et al. 2019; Yang et al. 2018).

Non-invasive sensors of UA in human urine have been recently developed (Bai et al. 2017; Yang et al. 2018), and various techniques, such as liquid chromatography (Li et al. 2015), capillary electrophoresis (Pormsila et al. 2009), and fluorescence spectroscopy (Azmi et al. 2018) have been used for UA detection. However, while these techniques offer high sensitivity and precision, they also have some limitations such as high cost, and the requirement of highly trained personnel. An electrochemical sensor offers an alternative approach owing to its simplicity,
miniaturization, rapid response and low cost (Liu et al. 2012; da Cruz et al. 2017; Income et al. 2019). Traditionally, the sensing of UA is performed via an enzymatic reaction using uricase. Although enzyme-based sensors provide high specificity and sensitivity, they have some major disadvantages, including high cost, operational complexity and low stability (Arora et al. 2014).

Non-enzymatic UA sensors based on oxidation processes occurring on electrodes have been developed (Income et al. 2019). However, non-enzymatic sensors require of high surface areas for accurate detection. Metal nanoparticles have been utilized for the surface modification of electrodes owing to their high conductivity, large surface area, high mechanical strength, and electrocatalytic properties (Yukird et al. 2018; Ji et al. 2018; Wang et al. 2018, 2016; Ruecha et al. 2017). Among the metallic nanoparticles, gold nanoparticles (AuNPs) have been widely applied for non-enzymatic sensors due to high biocompatibility and easy conjugation with cellulose (Yokota et al. 2008a; Van Rie and Thielemans 2017; Azetsu et al. 2011; Park et al. 2012; Lam et al. 2012; Kitaoka et al. 2011).

In this manuscript, we report on the development of an electrochemical sensor for the non-enzymatic detection of UA using cotton threads as flexible substrates. We coated the cotton threads with a conductive carbon ink and then electrodeposited AuNPs to increase the sensor’s surface area and its electrochemical sensitivity to UA. The performance of the sensor was systematically investigated and tested in real human urine samples.

Material and methods

Chemicals and materials

Uric acid (UA), potassium ferricyanide (K₃[Fe(CN)₆]), potassium ferrocyanide (K₄[Fe(CN)₆]) and gold (III) chloride trihydrate (HAuCl₄·3H₂O) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Silver/silver chloride (Ag/AgCl; C2130809D5) and carbon ink (C2030519P4) was obtained from Gwent group (Torfaen, United Kingdom). The carbon ink is composed of a solid carbonaceous component (e.g. carbon and graphite) and an epoxy resin (e.g. thermoplastic resin and some other additives). Potassium dihydrogen phosphate (KH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄), sodium chloride (NaCl), potassium chloride (KCl) and sulphuric acid (H₂SO₄) were obtained from Carlo Erba Reagenti-SDS (Val de Reuil, France). All solutions were prepared using purified water (Millipore, USA, R ≥ 18.2 MΩ cm⁻¹). A Phosphate buffered saline solution (PBS, 0.1 M pH 6.0 (Tukimin et al. 2017; Zhang et al. 2012; Chen et al. 2018; Jalalvand 2020; Lorenzetti et al. 2019; Rebelo et al. 2004)) was prepared by dissolving 1.42 g Na₂HPO₄, 1.36 g KH₂PO₄, 0.58 g NaCl and 0.74 g KCl in 100 mL of high purified water. A stock solution of UA was freshly prepared in 0.1 M PBS. All chemicals were used as received.

Apparatus

The morphology and elemental composition of the cotton thread sensor were characterized by scanning electron microscopy (SEM) with energy dispersive X-rays spectroscopy (EDX) (JSM-6400; Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan). Electrochemical measurements were performed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) modes on a CHI1240B (CH Instruments, Inc., Austin, TX, USA) potentiostat, controlled with CHI1240B software. Laser desorption/ionization mass spectrometer (LDI-MS; Autoflex III MALDI-TOF MS; Bruker, MA, USA) with 25% N₂ laser intensities at 337 nM and 40 shots was used to validate the concentration of uric acid in the samples.

Sensor design

The fabrication of the cotton thread sensor is shown in Scheme 1. Firstly, a cotton thread (DMC special 25, 100% cotton B5200) was soaked in the prepared carbon ink (C2030519P4), passed through a pinhole of a needle to remove the excess ink and obtain uniformed carbon coating on the thread, and baked at 60 °C for 30 min. The carbon-coated cotton thread was used as a working electrode (WE) for all experiments. To fabricate the counter and reference electrodes of an electrochemical sensor, carbon ink (C2030519P4) was screen-printed on a polyvinylchloride (PVC) substrate and heated at 60 °C for 30 min. After heating, Ag/AgCl ink (C2130809D5) was screen-printed on the same PVC substrate, dried at...
60 °C for 30 min, and used as a reference electrode (RE). After obtaining screen-printed electrode (SPE) template on PVC containing the RE and CE, a transparent film was punched in a hole diameter of 6 mm and attached on the as prepared SPE template using double-sided tape for creating electrochemical testing zone and hydrophobic barrier, which limit the sample area contacting with three electrodes on SPE. The carbon-coated cotton thread was placed between the RE and CE. Gold nanoparticles (AuNPs) were electrodeposited on the WE, by dropping 40 μL of 3.0 mM HAuCl₄ followed by the applied potential of −0.6 V (vs. Ag/AgCl) for 120 s (Charoenkittamorn et al. 2015). The AuNP modified cotton thread was carefully rinsed with purified water and dried at 60 °C for 1 h prior to use.

Characterization techniques

The surface morphology of the cotton thread, carbon-coated cotton thread and AuNP/carbon-coated cotton thread was observed using scanning electron microscopy (SEM). A dried sample was mounted directly on to a specimen stub, and imaging was carried out under high vacuum with an accelerated voltage of 10.0 kV using a magnification of 20,000×. The presence of functional groups in cotton thread, carbon-coated cotton thread and AuNP/carbon-coated cotton thread was verified by Fourier-transform infrared spectroscopy (FTIR: PerkinElmer, Spectrum One) with attenuated total reflectance (ATR) in a range of 4000–500 cm⁻¹.

Electrochemical measurements

Electrochemical measurements were performed on a potentiostat using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The cotton thread electrode was electrochemically characterized using a standard solution of 5.0 mM [Fe(CN)₆]³⁻/⁴⁻ via CV with a potential ranging from −1.2 to +0.9 V and a scan rate of 100 mV/s. During UA determination procedures, DPV measurements were performed using a potential from −0.3 to +0.8 V with a step potential of 15 mV, an amplitude of 200 mV and a pulse width of 50 ms.
Sample collection and sample preparation

A standard calibration curve, obtained by using with the spiking method, was selected for the detection of UA in human urine samples. Urine samples were collected from 5 different healthy adults as control measurements with a non-bias prior to medical diagnosis. The urine sample was diluted with 0.1 M of PBS solution (pH 6) in a ratio of 1:1. Known amounts of UA (0.5, 1.0 and 2.0 mM) were added to the diluted samples. 40 µL of the prepared samples were analyzed using the cotton thread sensor via DPV. The DPV results were compared to the values obtained with a LDI-MS (Autoflex III MALDI-TOF mass spectrometer; Bruker, MA, USA). For the LDI-MS analysis, 10 µL spiked samples were dropped on a stainless-steel MALDI-MS target plate and dried at room temperature (25 ± 5 °C) prior to analysis by LDI-MS.

The electrochemical sensor was also integrated into a diaper and used for the detection of UA in artificial urine. The artificial urine solution was prepared as described in a previous report (Hiraoka et al. 2020) with UA concentrations (1–3 mM).

Results and discussion

Physical characterization of cotton thread-based electrode

The surface of the cotton thread working electrode was characterized by scanning electron microscopy (SEM) with an energy dispersive X-rays spectroscopy (EDX) as shown in Fig. 1. SEM images display a smooth morphology for the uncoated cotton thread, whereas the carbon-coated cotton thread show higher surface roughness. To increase the specific surface area and electrical conductivity of the cotton thread, gold nanoparticles (AuNPs) were added via electrodeposition. SEM images show that the AuNPs were uniformly deposited on the carbon-coated cotton thread surface, with a particle size varying between 98.10 and 100.42 nm. EDX measurements were used to identify the characteristic peaks of Au as shown in Fig. 1d. The weight contents of C, O and Au were 74.59%, 1.59% and 23.82%, and the atom content of C, O and Au were 96.57%, 1.55% and 1.88%.

The FTIR spectra of uncoated cotton thread, carbon-coated cotton thread, and AuNP modified carbon-coated cotton thread were demonstrated in Fig. S1. The absorption bands of the cotton thread were observed at 3332 cm⁻¹ (O–H stretching), 2891 cm⁻¹ (C–H asymmetric stretching), 1715 cm⁻¹ and 1651 cm⁻¹ (C=O stretching of a carboxylic acid and ester). The intense peak at 1023 cm⁻¹ was possibly caused by overlapping bands of the functional groups of polysaccharides in cellulose. In addition, a small peak at 894 cm⁻¹ was a characteristic of β-glycosidic linkages of cellulose monosaccharides (Portella et al. 2016; Krishnamoorthy et al. 2012; Xu et al. 2018; Alamer 2018). Moreover, the spectra of the carbon-coated cotton thread and AuNP/carbon-coated cotton thread were similar to the uncoated cotton thread but lower absorption intensity due to the physical adsorption of carbon ink and AuNPs on the cotton thread surface.

Fabrication of the modified cotton thread-based sensor

A two-step fabrication was carried out to create the AuNP/carbon-coated cotton thread working electrode. Initially, cotton threads were soaked in carbon ink and passed through a pinhole to create the uniformed coating on the cotton thread’s surface and remove the excess amount of the carbon ink. HAuCl₄ was electrodeposited to form AuNPs on the carbon-coated cotton thread. Several parameters affecting the electrochemical performance of the sensor were optimized, such as the HAuCl₄ concentration and the electrodeposition time measuring by DPV in 1.0 mM UA.

The effect of HAuCl₄ concentration on the sensitivity of the sensor was systematically investigated as shown in Fig. 2a, b. Five different concentrations of HAuCl₄ were electrodeposited on the surface of the sensor at a deposition potential of − 0.6 V for 120 s. The results show that the anodic peak current response increased with the increasing concentration of HAuCl₄, and the highest anodic peak current was obtained at a concentration of 3.0 mM. After reaching the maximum, the anodic current responses decreased for concentrations higher than 3.0 mM due to the higher density of gold cluster causing the agglomeration on the thread’s surface (Fig. S2). Agglomeration leads to a decreased surface area and smaller
The electrochemical response of the system (Perez et al. 2011; Kuriganova et al. 2020; Li et al. 2019b; Kimuam et al. 2020; Ahluwalia et al. 2014). We selected a concentration of 3.0 mM HAuCl$_4$ for the modification of the thread-based sensor.

The effect of electrodeposition time was also investigated in a range between 30 and 240 s. As shown in Fig. 2c, d, the anodic peak current increased when the electrodeposition time increased, and the highest anodic peak current was obtained at 120 s of deposition time. After the maximum is reached, we found that the anodic peak current response decreased with increasing electrodeposition time. Longer electrodeposition times mean denser gold clusters (Fig. S3) hence leading to the decreasing of surface area (Shu et al. 2014; Dominguez Renedo and Arcos Martinez 2007; Charoenkitamorn et al. 2015; Majid et al. 2006). The electrodeposition time of 120 s was chosen for further experiments.

Electrochemical characterization of the sensor

The electrochemical behavior of the carbon-coated cotton thread, with and without AuNPs, was investigated by cyclic voltammetry (CV), using 5.0 mM [Fe(CN)$_6$]$^{3-}/4-$ in 0.1 M KCl as shown in Fig. 3a. In the cyclic voltammograms, the presence of AuNPs on the carbon-coated cotton thread (green line), showed higher anodic and cathodic peak currents towards [Fe(CN)$_6$]$^{3-}/4-$ detection, which was approximately 2 times higher than the current for the thread absent of AuNPs (pink line). The incorporation of AuNPs on the surface improves both the specific surface area and the electrochemical conductivity of the sensor, hence leading to improved sensitivity.

To assess the analytical performance of the sensor, 1.0 mM UA was measured using differential pulse voltammetry (DPV) as shown in Fig. 3b. The anodic peak current response for UA measured on the AuNP/carbon-coated cotton thread (yellow line) significantly increased compared to the one measured on the carbon-coated cotton thread (blue line). These
results indicated that the presence of AuNPs can enhance the electrochemical response for UA detection. Electrochemical impedance spectroscopy (EIS) was also used to confirm the enhanced sensitivity of AuNP modified cotton thread-based electrodes. As shown in Fig. 3c, the smallest semicircle was observed for the AuNP modified cotton thread-based sensor. The low charge transfer resistance occurred because the AuNP modified electrodes (blue) can effectively accelerate the electron transfer through cotton thread-based electrode surface (Huang et al. 2007; Motia et al. 2020; Bhavani et al. 2019; Wang et al. 2018; Benvidi et al. 2015). The cotton thread-based sensor (orange) shows the higher $R_{ct}$ value which indicated a higher electron transfer resistance, hence indicating that the AuNP coated electrode was a suitable working electrode for UA detection.

Optimization of parameters for uric acid detection

To obtain the highest electrochemical sensitivity of the developed sensor for UA, electrochemical parameters such as step potential, amplitude and pulse width were systematically optimized when measuring 1.0 mM
UA in 0.1 M PBS. The selection of optimal conditions was based on the highest current response along with the smaller peak width and high peak symmetry. Figure S4 shows that the highest anodic peak current response at 15 mV of step potential, an amplitude of 200 mV, and a pulse width of 50 mV. At higher values of step potential, amplitude and pulse width, the anodic peak current response decreased due to broader peak shape. Therefore, in this study the above-mentioned conditions were selected for the detection of UA.

The analytical performance of the cotton thread-based sensor

The analytical performance of the AuNP modified cotton thread-based sensor on the detection of UA was investigated via DPV. As shown in Fig. 4, the anodic peak current linearly increased with UA concentration in the range between 10 μM and 5.0 mM (Fig. 4a). Two linear ranges of UA were observed from 10 μM–0.5 mM (Fig. 4b) and 0.5–5.0 mM (Fig. 4c) with a correlation coefficient (R²) of 0.9975 and 0.9987. The limit of detection (LOD) was calculated using the...
following equation: $\text{LOD} = 3S/N$, where $S$ is a standard deviation of a signal obtained from a blank solution ($n = 7$), and $N$ is a slope of calibration curve (Cheng and Kao 2016; Mukdasai et al. 2016; Yang et al. 2017; D’Souza et al. 2021). The LOD of AuNP modified cotton thread-based sensor was found to be 0.12 $\mu$M, which was calculated from the low concentration range (10 $\mu$M–0.5 mM) of calibration curve (Fig. 4b) due to lower LOD.

A linear range of 0.1–2.0 mM was obtained when Au NPs were not present on the surface of the electrode (Fig. S4) which is a narrower range than that of 10 $\mu$M to 5.0 mM for the sensor coated with AuNPs (Fig. 4). These results confirm that the presence of AuNPs improves the sensor’s signal linearity possibly due to the enhanced surface area and conductivity of the AuNPs. The slope of the calibration curve obtained from the sensor coated with AuNPs was higher than the slope for the sensor without AuNPs (Fig. S5), suggesting that the enhanced sensor sensitivity results from the presence of AuNPs. Interestingly, LOD of the carbon-coated cotton thread-based sensor was found approximately 13 times higher than the sensor with AuNPs (0.12 $\mu$M). These results indicate that the presence of AuNPs on the electrode’s surface drastically improved the sensitivity and LOD of the sensor.

Our sensor has a lower LOD and wider linear range than those previously reported as summarized in Table 1.
Interference study

The anti-interference ability of the cotton thread-based sensor was assessed by adding potential interfering substances such as a tenfold excess of glucose, creatinine, KCl, NaCl, urea, and a onefold excess of ascorbic acid and 1% excess of BSA. As shown in Fig. 5, there was no significant changes in the current measurements as the result of the presence of interfering substances (Lee et al. 2017; Jia et al. 2011; Zhu et al. 2017; Baig and Kawde 2016; Du et al. 2013).

Stability of the cotton thread-based sensor

The stability of the cotton thread-based sensor was investigated by measuring the DPV response to 1.0 mM of UA in PBS solution multiple times. The sensors were stored at room temperature for 30 days.
and the current responses remained above 96.48% of their initial values as shown in Fig. S6. These results are evidence of the stability and reproducibility of our sensors (Taei et al. 2016; Chang et al. 2014; Hu et al. 2008; Kannan and John 2009; Tan et al. 2020).

Real—life sample application and method validation

To confirm the potential application of the cotton thread-based sensor in complex biological matrices, pooled urine serum samples were collected from 5 healthy adults (female) to represent non-bias errors prior to medical diagnosis. Each urine sample was diluted with 0.1 M PBS solution, pH 6.5 (1:1). The cotton thread-based sensor was used to detect UA in the samples via a standard addition method. A known amount of UA (0.5, 1.0 and 2.0 mM) was added to the diluted samples. The analytical results are summarized in Table 2. The percent recovery ranged from 100 to 108 with a relative standard derivation (RSD) of less than 6.0% (n = 3). These results were validated by comparing these measurements with those obtained with a standard LDI-MS method as shown in Table 2.

Table 2  Determination of UA in human urine samples using the modified cotton thread-based sensor and LDI-MS (n = 3)

| Spiked UA concentration (mM) | Modified cotton thread-based sensor (mM) | Standard LDI-MS (mM) | Recovery (%) |
|-----------------------------|----------------------------------------|----------------------|-------------|
| Blank                       | 0.61 ± 0.08                            | 0.70 ± 0.09          | –           |
| 0.5                         | 1.11 ± 0.02                            | 1.17 ± 0.05          | 100         |
| 1.0                         | 1.69 ± 0.05                            | 1.73 ± 0.07          | 108         |
| 2.0                         | 2.77 ± 0.12                            | 2.84 ± 0.15          | 108         |

The cotton thread-based electrochemical sensor was also used to detect UA in artificial urine media. As shown in Fig. S7, a linearity was found in a range of 0.4–3.4 mM for UA in artificial urine samples. The sensor threads were directly integrated into a diaper and used to measure UA in artificial urine poured over the diaper. Known concentrations of UA (1.0, 2.0 and 3.0 mM) were added to the artificial urine media, and 50 mL of the solution were poured into the diaper. Table S1 summarizes the recovery percentages which ranged from 96 to 105 with an RSD of less than 9% (n = 3), verifying the high accuracy of the sensor in the diaper.

Conclusions

An electrochemical sensor for the non-enzymatic detection of UA, was fabricated using cotton threads coated with AuNPs. The cotton thread-based sensor exhibited high sensitivity to UA. The sensor shows a wide linear detection range of 10 μM–5.0 mM with a low detection limit of 0.12 μM. This enzyme-free sensor exhibits long-term stability, anti-interference ability, and satisfactory recoveries for detecting UA in human urine samples. The sensor was effectively applied for differentiation between a patient diagnosed with gout and a normal individual with a required cut off value (uric acid ≥ 4.46 mM). This sensing platform was successfully integrated into a diaper for direct detection of UA in artificial urine, enabling its potential for gout disease screening.

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Declarations

Conflict of interest  The authors declare no conflict of interest.

Ethical standard  All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee from Chulalongkorn Hospital.

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