Biosensors and Bioelectronics 132 (2019) 352–359

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A low-cost, portable and easy-operated salivary urea sensor for point-of-care application

Kuan-Hsun Wang[a], Ju-Chun Hsieh[a], Chang-Chiang Chen[b,c,**], Hsiao-Wen Zan[a,*], Hsin-Fei Meng[d], Sheng-Yu Kuo[e], Minh Trâm Ngọc Nguyễn[d]

[a] Department of Photonics, College of Electrical and Computer Engineering, National Chiao Tung University, 1001, Ta Hsueh Rd., 300 Hsinchu, Taiwan
[b] Department of Biological Science and Technology, National Chiao Tung University, 1001, Ta Hsueh Rd., 300 Hsinchu, Taiwan
[c] Department of Internal Medicine, Division of Nephrology, National Taiwan University Hospital Hsin-Chu Branch, 25, Ln. 442, Section 1, Jingguo Rd., 300 Hsinchu, Taiwan
[d] Institute of Physics, National Chiao Tung University, 1001, Ta Hsueh Rd., 300 Hsinchu, Taiwan
[e] Institute of Biomedical Engineering, National Chiao Tung University, 1001, Ta Hsueh Rd., 300 Hsinchu, Taiwan

ARTICLE INFO

Keywords:
Non-invasive
Salivary urea
Real-time
Optical fiber
Point-of-care

ABSTRACT

Salivary urea was reported to be a useful biomarker to reflect the blood urea nitrogen in chronic kidney disease patients. However, as a new biomarker, enormous clinical trials are required to define the intended-use and to verify the specification. In this report, we demonstrated a low-cost easy-operated real-time sensing system (optical fiber-urea-sensing, OFUS, system) to detect salivary urea. We aim to make the system easily reproduced by the community to stimulate abundant clinical tests worldwide. The OFUS system is composed of a simple three-dimensional printed tank to link with two optical fibers, one connecting with a commercial light-emitting diode to deliver the input light signal, the other connecting with a commercial cadmium sulfide photo-conductive cell to detect the sensing signal. To allow on-site detection without any sample pretreatment, only 1 μl saliva is needed to be mixed with 10 μl urease solution and 90 μl pH indicator solution in the reaction tank and the detection time is only 20 s. A stable and reproducible calibration curve can be easily built with a detection range as 24–300 mg/dL. The OFUS system successfully detected saliva with added synthetic urea and samples from chronic kidney disease patients. A good agreement between the OFUS system and the commercial kit was obtained. A good correlation between salivary urea and the blood urea nitrogen was also confirmed.

1. Introduction

Non-invasive bio-medical sensors are emerging technologies in the coming era of cloud medicine with internet-of-things (IoT) facilities. Clinical studies found that many important bio-markers may exist in human breath (Chuang et al., 2017; Davies et al., 2014; Di Natale et al., 2014; Kumar et al., 2015; Mathew et al., 2015; Saalberg and Wolff, 2016; Wilson, 2015), urine (Clinton and Lotan, 2017; Lopez-Giacoman and Madero, 2015; Park et al., 2017; Won et al., 2016; Zheng et al., 2015), saliva (Ahmed et al., 2015; Celec et al., 2016; Nagler, 2008; Pallos et al., 2015; Prasad et al., 2016; Shirzaiy et al., 2015), and tear (Badugu et al., 2018; Farandos et al., 2015). The importance to develop the in-vitro bio-marker detecting is hence rapidly growing. Saliva has been reported to be an informative in-vitro liquid. In the cases approved by Food and Drug Administration (FDA), saliva detecting is useful for the fast screening of drug taking (Drugs of Abuse (Collection Kit)) and the human immunodeficiency virus (HIV) infection (First Rapid Home-Use HIV Kit Approved for Self-Testing). Lately, the deoxyribonucleic acid (DNA) analysis from saliva sample is also an emerging business (FDA allows marketing of first direct-to-consumer tests that provide genetic risk information for certain conditions). Detecting saliva cortisol to indicate stress reaction is also recognized by FDA and European Conformity (CE) (Diagnostic Salivary Progesterone Elisa Kit (FDA, CE Mark)).

In recent studies, salivary urea testing is also developed to reflect the blood urea nitrogen (BUN) in chronic kidney disease (CKD) patients. As many countries are facing the aged society, CKD is one of the major diseases affecting human health. Approximately 30 million

* Corresponding author.
** Corresponding author at: Department of Internal Medicine, Division of Nephrology, National Taiwan University Hospital Hsin-Chu Branch, 25, Ln. 442, Section 1, Jingguo Rd., 300 Hsinchu, Taiwan.
E-mail addresses: cck85515@gmail.com (C.-C. Chen), hsiaowen@mail.nctu.edu.tw (H.-W. Zan).

https://doi.org/10.1016/j.bios.2019.03.007
Received 20 December 2018; Received in revised form 5 March 2019; Accepted 6 March 2019
Available online 07 March 2019
0956-5663/

Americans, 14.8% of the American population, have CKD (Saran et al., 2018), but most of them have only a little awareness of this disease. Patients with hypertension or diabetes are at high risk for CKD. Dialysis or kidney transplantation is a treatment for severe kidney failure. Early detection and early treatment may slow the progression of CKD. BUN is one of the biochemistry parameters to evaluate kidney function. Hence, it would be beneficial to the assessment of kidney function if the BUN test can be done by a non-invasive approach, such as salivary urea test. Several previous studies showed a good correlation between salivary urea and blood urea as listed in Table S1 (Ahmed et al., 2015; Akai et al., 1983; Cardoso et al., 2009; Pandya et al., 2016; Sein, Arumainayagam, 1987; Tomás et al., 2008). When salivary urea may be a useful indicator of kidney function, the development of the salivary urea sensor as a non-invasive device is also essential.

Currently, salivary urea sensing tools are mostly based on the commercial kit with absorption spectrometer readout (Ahmed et al., 2018; Govindarajan et al., 2017; Piccione et al., 2006; Renda, 2017; Romero et al., 2016). With urease as the enzyme to ensure the sensing selectivity, the sensing mechanism mostly relies on the hydrolysis of urea to generate ammonia and to change the color of pH indicator. Some strips are developed for eye recognition and for smartphone reading to promote the point-of-care (POC) applications (Akai et al., 1983; Alev-Tuzner et al., 2018; Calice-Silva et al., 2014; Evan et al., 2017, 2018; Raimann et al., 2011, 2016; Soni et al., 2018). For example, Alev-Tuzuner et al. used polyethyleneglycol (PEG) based hydrogel to entrap urease and then allocated the hydrogel onto a pH-indicator paper to form the strip. The strip determines the urea concentration ranged from 20 mg/dL to 200 mg/dL in 1 min (Alev-Tuzner et al., 2018). In addition to eye recognition, smartphone-based sensing systems have been proposed to read the color change of the strips (Lopez-Ruiz et al., 2014; Oncescu et al., 2013; Soni et al., 2018). In 2018, Soni et al., developed a smartphone reading system to read the color changing signal on a polyvinyl alcohol (PVA) based hydrogel sensing strip. They successfully determine salivary urea with concentration ranged from 10 to 260 mg/dL in 20 s (Soni et al., 2018). The fast response and the easy read-out using smartphone facilitate the future applications for end-users. However, there is still a big gap between the clinical studies and the real medical tool with FDA approval. Since salivary urea is still a new bio-marker, the commercialization of medical equipment for saliva urea faces challenging. The missing link is the recognition of this bio-marker for clearly specified intended-use. Either to be used as fast screening, as protein intake monitoring, or as the disease progress alerting, more abundant clinical evidence is required. However, to stimulate clinical trials in clinical centers, the results in prior works have some limitations. The development of a hydrogel-based sensing strip is important when facing end-user for future commercialization. For doing human trials in clinical centers, however, it is not possible for doctors and nurses to spend efforts fabricating these new strips by themselves. Considering the requests in doing human trials, we noticed that the barrier to study salivary urea relied on the following factors. (1) Difficult sample storage. (2) Lack of easy, portable, fast, and low-cost testing. (3) Lack of easy and fast calibration. If the above barrier can be lowered, the clinical study for salivary urea can be done more easily and the POC application may be realized.

Generally, saliva samples face the difficulty of storage without affecting by environmental bacteria. In most cases, frozen storage is needed (Piccione et al., 2006; Renda, 2017; Romero et al., 2016). In some prior reports, for example when doing the DNA analysis, saliva samples can be stored at room temperature by adding additional buffer solution to stabilize the DNA (Slowey, 2013; Streckfus, 2015). However, such method is not yet verified for salivary urea. An alternative method to solve the difficulty of sample storage is doing “on-site” immediate analysis after collecting the samples.

To realize the “on-site” salivary urea detection, a low-cost, portable, easy-operated saliva urea testing system is in need. Hence, in this work, we perform an optical-fiber-urea-sensing (OFUS) system by using a simple three-dimensional (3-D) printed reaction tank to link with two optical fibers, one connecting with a commercial light-emitting diode (LED) to deliver the input light signal, the other connecting with a commercial cadmium sulfide (CdS) photo-conductive cell to detect the sensing signal. Between the two optical fibers, the salivary sample is mixed with the urease solution and pH indicator solution to deliver the sensing reaction. The designed structure does not require any dedicate optical align between the two optical fibers. We apply ultraviolet (UV) glue to enhance the light coupling between the optical fibers and the LED/photo-conductive cell. Then, by changing urea concentration in the prepared sensing solution, we demonstrate a stable calibration curve with high reproducibility and good repeatability. We also demonstrate the on-site real-time detection by using real saliva with added synthetic urea. Compared with the commercial kit, our proposed method delivers a very good match. Finally, we used the OFUS system to detect saliva samples collecting from patients with chronic kidney diseases. A good correlation between salivary urea and the blood urea nitrogen is obtained. We confirm that the proposed low-cost system realizes the on-site testing with easy operation. Moreover, to let the OFUS system easily adopted by the community and hence to speed up the worldwide studies on salivary urea, the OFUS system has no proprietary and is highly replicable. The purpose of this study is to stimulate abundant clinical studies, hopefully realizing the POC applications with a clear intended use in the near future.

2. Experimental section

2.1. Materials

Urea, urease (U4002 - 20KU), and bromothymol blue (BTB) powders were purchased from Sigma-Aldrich. The multimode optical fiber was purchased from Thorlabs (FT200UMT). The reaction tank was constructed using a 3-D printer.

2.2. Preparation of synthetic urea solution, urease solution, and bromothymol blue solution

Urea powder was dissolved in deionized (DI) water to prepare concentration range from 24 to 300 mg/dL. Urease solution was prepared by dissolving 2.1 mg urease powder in 10 ml DI water. The BTB pH indicator was prepared by dissolving 0.1 g BTB powder in 4.3 ml of 0.05 M NaOH. DI water was added to the mixed solution to obtain a final volume of 200 ml and the solution was diluted 10-fold using DI water. The pH value of BTB before testing is fixed at 7.4.

2.3. Sensing system

The OFUS system (Fig. 1) consisted of a 585 nm yellow LED, an input optical fiber, a 1 cm × 0.5 cm × 0.7 cm 3-D printing reaction tank, an output optical fiber, a CdS photo-conductive cell (SEN5003), a hot plate, and an I-V analyzer to measure electric signals. The photo-conductive cell needed to be shielded from the ambient light. The LED also needed to be covered to ensure that the LED light entered the tank only through the input optical fiber. The photos of the OFUS system, the top view and the side view of the tank were also shown in Fig. 1. The optical fibers, stained with blue color in the photos, were put into the grooves at the side-wall of the tank. Then, polydimethylsiloxane (PDMS) films were inserted into the grooves to avoid the liquid leak. The input optical fiber delivered light to the reaction tank. The silica core diameter of the optical fiber was 200 ± 5 μm. The hard polymer cladding diameter of the optical fiber was 225 ± 5 μm. The buffer coating diameter of the optical fiber was 500 ± 30 μm. The numerical aperture (NA) of the optical fiber was 0.39. To easily connect the optical fibers and the LED, reaction tank, and photo-conductive cell, the buffer coating at both ends of the optical fiber were removed by the wire stripper. One end of the input optical fiber was fixed on the LED by

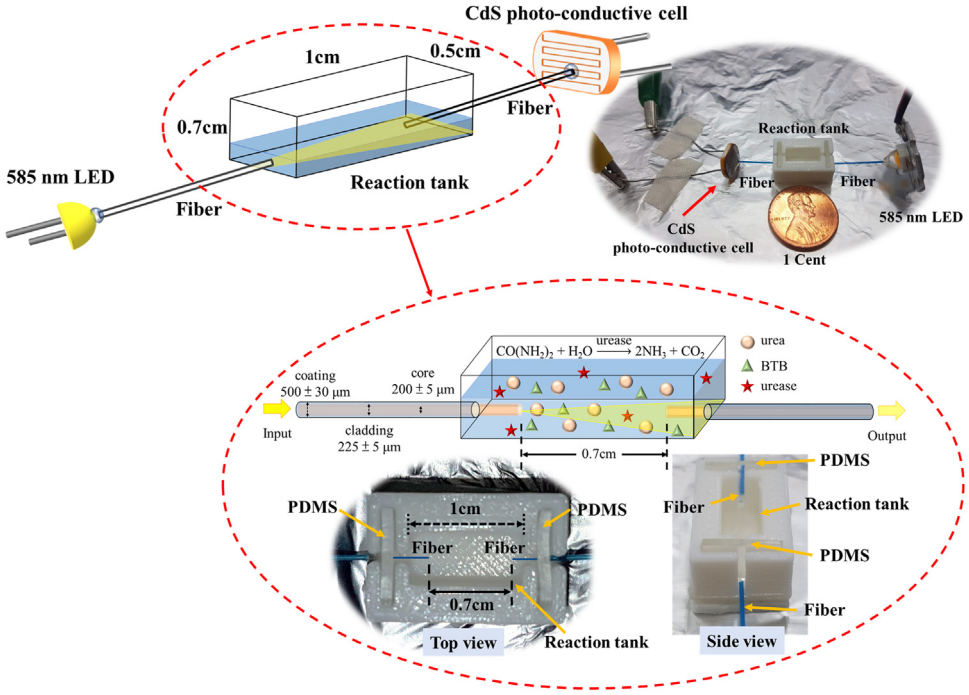

Fig. 1. The schematic diagram and the photos of the OFUS system. In the photos, the optical fibers were stained with blue color and were put into the grooves at the side-wall of the tank. The PDMS films were used to avoid the liquid leak.

UV glue, which improved the transmission of light from LED into the optical fiber, and the other end was connected to the bottom of the reaction tank. The output optical fiber, which was connected to the photo-conductive cell by UV glue, was used to collect light signal to the photo-conductive cell detector. The end-end distance between the two optical fibers was typically fixed at 0.7 cm in this study. The designed structure did not require any dedicate optical align between the two optical fibers. The temperature was stabilized at 37 °C with the help of a hot plate. The current of the photo-conductive cell was measured using the Keysight U2722A modular source measure unit when the cell was biased at 3 V.

2.4. Urea detection

Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide (Eq. (1)). Subsequently, ammonia reacts with water to form ammonium ($NH4^+$) and hydroxyl (OH^-) ions (Eq. (2)), which increases the pH value. We used a colorimetric pH indicator, BTB, to detect the change of pH and hence to determine urea concentration. The schematic of the process for urea detection is presented in Fig. S1. First, 90 μl BTB was added to the reaction tank (Fig. S1(a)) to completely cover the two optical fibers. Then, 1 μl urea solution was added to the reaction tank and was stirred to form a uniform solution (Fig. S1(b)). Then, 10 μl urease solution was added into the mixture and was stirred for 2 s (Fig. S1(c)). It is worth mentioning that, to avoid the interference from the corners of the rectangular tank and to quickly obtain a uniform solution, we always add the solution to the position in the middle of the two optical fibers. We didn't use a cylindrical tank because the surface roughness was greatly increased when using a 3-D printer to fabricate cylindrical shape. Poor surface roughness may cause difficulty in cleaning. However, in the future, if the molding process is used, the cylindrical shape may be preferred to avoid the corner effect. When the color in the solution changed with time, the urea concentration was measured by reading the current change of the photo-conductive cell. After finishing the measurement, we removed the mixed solution with the pipette. To reuse the reaction tank for the next testing, the reaction tank was rinsed three times with 0.2 ml DI water (Fig. S1(d)).

$$CO(NH_2)_2 + H_2O \xrightarrow[\text{urease}]{} 2NH_3 + CO_2 \quad (1)$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (2)$$

2.5. Calibration curve of the urea concentration

Prior to measure the saliva sample, we build the calibration curve by using the OFUS system to detect the synthetic urea solution with urea concentration ranging from 24 to 300 mg/dL. Follow the process as shown in Fig. S1, we define the current variation ratio at the measurement time of 20 s to be the sensing response. The response is calculated by Eq. (3). Then, the calibration curve is defined as the sensing response as a function of urea concentration at a fixed measurement time as 20 s.

$$\text{Response} = \frac{\Delta I}{I_0} \quad (3)$$

Where ΔI is the current variation within a fixed sensing time, I_0 is the initial current amount.

To study the interference factors of the OFUS system, we changed the pH value of the urea solution, the ambient light intensity, the misalignment of the optical fiber ends, and the end-end distance between the two optical fibers to check their influences on the sensing response. The discussion will be given in a later section.

2.6. Saliva sampling and measurement

To evaluate the function of the OFUS system, we collected saliva samples from both healthy individuals and CKD patients.

For samples obtained from healthy individuals, we added synthetic urea to obtain higher urea concentrations to simulate the conditions in CKD patients. Soon after collecting the saliva and mixing the saliva with the synthetic urea, these samples were immediately tested by the OFUS system. Because we only need 1 μl of saliva sample for testing, we do not need any pretreatment such as centrifugal filtration or freezing of the saliva samples. The easy sample handling and the immediate testing verify the on-site real-time sensing function of the OFUS system. By

changing the urea concentration from 24 to 180 mg/dL, the sensing response is then plotted as a function of urea concentration to serve as the calibration curve of the OFUS system. To compare the OFUS system with the commercial kit, the same saliva samples were also measured by the commercial urea assay kit (QuantiChrom™ Urea Assay Kit, DIUR-100, BioAssay Systems, Hayward, CA, USA).

Saliva samples from the CKD patients and the healthy individuals were collected as the following procedures. Patients with kidney disease were instructed to fast for at least 8 h before saliva sampling. Saliva collection was done between 8 a.m. and 11 a.m. The unstimulated saliva was collected by spitting into a sterile plastic bottle and then was stored at −80 °C until salivary urea analysis was performed. Prior to analysis, each saliva sample was defrosted in a sterile laminar flow hood at room temperature for 1 h. The samples were used directly without centrifugal filtration because the pipette easily accommodated 1 μl of clear saliva from the saliva sample. After measuring the sample by the OFUS system, the urea concentration was estimated by the calibration curve.

2.7. Analysis of blood urea nitrogen

The BUN was measured by urea nitrogen reagent kit (WAKO, UN L-Type S), done by clinical chemistry analyzer (Hitachi Module Assembly Type Automatic Analyzer 7180A), at the National Taiwan University Hospital Hsin-Chu Branch.

3. Results and discussion

The real-time sensing current for 180 mg/dL synthetic urea solution is shown in Fig. 2(a). Hydrolysis of urea by urease commenced after addition of urea solution to the mixture, which already contained BTB and urease, followed by stirring for 2 s. The pH value, assessed using BTB as a pH indicator, also increased over time, which changed the color of the solution from green to blue; moreover, the absorbance at 585 nm increased (Fig. S2). The output light intensity decreased, as detected by the photo-conductive cell. This decrease was reflected in the current change. Specifically speaking, when the sensing time changes from 0 s to 20 s, the current decreases from 2.50 μA to 1.64 μA (Fig. 2(a)). The microampere output current indicates that the current signal can be easily read by a general current meter or by a low-cost circuitry.

The real-time sensing response for 180 mg/dL synthetic urea solution is shown in Fig. 2(b). The response was the current variation ratio calculated by Eq. (3). We normalized the current difference with its initial current level to reduce the interference of initial light intensity. It is noted that using the current variation ratio, instead of the current level, to represent the sensing response is important. The current level has a run-to-run variation because the light intensity may slightly vary from day to day. Using current variation ratio to serve as sensing response can greatly suppress such interference. The discussion will be given in a later section. When increased the urea concentration from 24 to 300 mg/dL as in Fig. 2(c), the response also increased due to the elevated concentration of ammonia, and hence the increase of pH value. The measurement time in Fig. 2(c) is 60 s for each urea concentration. We observed that the current changes significantly in the first 20 s of the measurement time (Fig. S3), as a result, we chose 20 s as a fixed sensing time to determine the urea concentration. It is noted that the commercial kit requests 20 min to reach the saturation and to give the measurement results.

To confirm that the OFUS system delivers fast operation and good repeatability, we performed the sequential testing as shown in Fig. 2(d). The sensing response of the 180 mg/dL urea solution was measured for three repeated times (blue regions). Between every measurement, the mixed solution was removed with the pipette and the reaction tank was cleaned using 0.2 ml DI water for three times. The cleaning process took about 104–117 s, as shown in the gray regions of Fig. 2(d). The total time (measuring time + washing time) for one test was approximately 200 s. Moreover, these three measurements exhibited almost identical response curve, indicating that the OFUS system delivers good repeatability and fast operation.

To carefully confirm the measurement repeatability, three urea concentrations (60 mg/dL, 180 mg/dL, and 300 mg/dL) were tested for three times as shown in Fig. 2(e). With a fixed sensing time as 20 s, the response of 60 mg/dL, 180 mg/dL, and 300 mg/dL were -0.11 ± 0.005, -0.33 ± 0.012, and -0.48 ± 0.012, respectively. The average and the standard deviation were calculated by using the three times measurement results. Apparently, we obtained a quite repeatable and reliable sensing response. In the insets of Fig. 2(e), we also put the photo image of the three samples (60 mg/dL, 180 mg/dL, and 300 mg/dL) at 20 s sensing time. It is worth noted that, by eye reading, the colors of the three conditions are not obviously different. We verify that the OFUS system can read the color change more precisely than the eye reading, within just 20 s sensing time. With the fixed sensing time as 20 s, we then constructed three calibration curves for the OFUS system in the range of 24 – 300 mg/dL (Fig. 2(f)). The first and the second calibration curves are measured on the same day with a time gap of 4 h. The third calibration curve is measured on a different day. Good agreement can be found between the three calibration curves, verifying that the OFUS system delivers stable sensing signals from time to time. Also, a good linearity was obtained for the three calibration curves.

We can use the standard deviation of the y-residual of regression lines to evaluate the limit of detection (LOD) of the three calibration curves in Fig. 2(f) (Shrivastava and Gupta, 2011). For urea concentration ranged from 24 to 300 mg/dL, the LOD values of the 1st, 2nd, and 3rd calibration curves are 25.5 mg/dL, 28.2 mg/dL, and 41.5 mg/dL, respectively. If reducing the detecting range to be 24–180 mg/dL, the LOD values of the 1st, 2nd, and 3rd calibration curves are 16.9 mg/dL, 18.5 mg/dL, and 17 mg/dL, respectively. For the 1st calibration curve, the intra-day relative standard deviation (RSD) values for urea concentration as 24, 60, 180, 300 mg/dL are 12.1%, 8.5%, 7.1%, and 2.5%, respectively. The resulting performance of the proposed OFUS system (the 1st curve) is compared with the prior works in Table 1. The RSD values are comparable to other prior works, while the OFUS system offers the on-site real-time testing with only 1 μl saliva without pretreatment. It is noted that the LOD value of OFUS system is 16.9 mg/dL when the linear range is 24–180 mg/dL. The LOD increases to be 25.5 mg/dL when extending the linear range to be 24–300 mg/dL. The inferior LOD in large dynamic range is because that, in high urea concentration, the color change of the BTB solution gradually saturates to degrade the sensing resolution. On the other hand, in low urea concentration, the response is too small. In the future, to improve the LOD, it is suggested to prepare two sensing solutions. One with a low BTB concentration to avoid saturation and to probe high urea concentration regime. The other has a high BTB concentration to increase the response in low urea concentration regime.

To further investigate the interference factors of the proposed system, we changed the following factors to study their influences on the calibration curves. Firstly, we changed the pH value of the urea solution by adding different concentration of hydrogen chloride solution or sodium hydroxide solution. As shown in Fig. S4(a), with fixed urea concentration as 180 mg/dL, increasing the pH value from 5, 6, 9, to 10 had only little influence on the sensing response. Different initial pH values changed the initial color in the sensing solution and hence the initial current. As previously mentioned, the response is the current variation ratio, not the current level. Hence, different initial current levels caused almost no influence on sensing response. We used three urea concentrations (60, 180, and 300 mg/dL) to check the pH value effect as in Fig. S4(b). We noticed that only pH 10 with high urea concentration caused an obvious deviation. Thankfully, in saliva, the buffer phenomenon confined the pH value. So far there was no report for saliva to have pH value as 10.

Next, we studied the interferences due to the ambient light and the

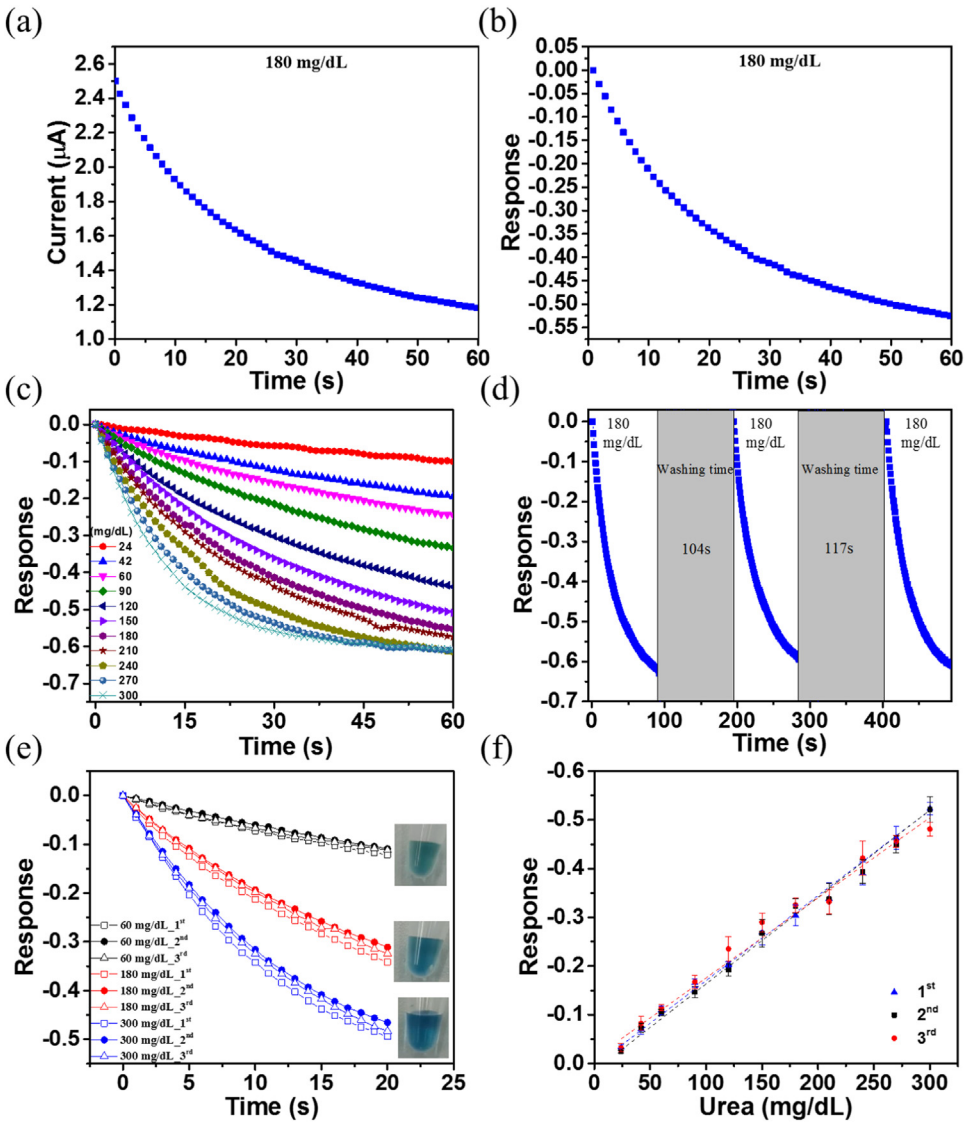

Fig. 2. (a) Real-time sensing current for 180 mg/dL synthetic urea solution. (b) Real-time sensing response for 180 mg/dL synthetic urea solution. (c) Real-time sensing responses from 24 mg/dL to 300 mg/dL. (d) Real-time sensing response measurement of the OFUS system. The sensing response of the 180 mg/dL urea solution was measured for three repeated times (blue regions). The sensing time was 90 s. The gray region is time taken to wash the reaction tank with DI water for three times. The average washing time was approximately 110 s (e) Repeatability of the OFUS system for 60 mg/dL (black), 180 mg/dL (red), and 300 mg/dL (blue). (f) Plotted three calibration curves for the OFUS system in the range of 24 – 300 mg/dL at 20 s sensing time.

misalignment of the two optical fibers. As shown in Fig. S5, with urea concentration as 180 mg/dL, the sensing response in dark and in ambient light (illuminance as 952 lx) were almost the same. This confirmed again that using current variation ratio suppressed the interference due to the light intensity change. Then, we intentionally shifted and tilted the light-receiving optical fiber. The shifting distance was about 1 mm and the tilting angle was about 30 degree. With identical ambient light, the systems with the aligned optical fibers and

Table 1
Salivary urea sensors.

Type	Volume	Linear range	Limit of detection	Response time	Intra-day relative standard deviation	Reference
Saliva urea nitrogen dipstick (Integrated Biomedical Technology)	50 μl	5–75 mg/dL	NR	1 min	NR	Evans et al. (2017)
Smartphone based urease-phenol red strip	5 μl	10–260 mg/dL	10.4 mg/dL	20 s	18.17 mg/dL: 9.9%	Soni et al. (2018)
PEG-based hydrogel-coated test strip	30 μl	20–200 mg/dL	20 mg/dL	1 min	20 mg/dL: 12.95% 80 mg/dL: 6.94% 200 mg/dL: 3.37%	Alev-Tuzner et al. (2018)
Amperometric enzyme electrode	NR	0.06–1.2 mg/dL	0.06 mg/dL	4 min	NR	Bertocchi and Compagnone (1996)
Test-strip	50 μl	NR	NR	15 min	16.3 mg/dL: 5.9% 26.3 mg/dL: 8.0% 46.8 mg/dL: 7.8%	Akai et al. (1983)
Optical fiber-urea-sensing (OFUS)	1 μl 1 μl	24–300 mg/dL 24–180 mg/dL	25.5 mg/dL 16.9 mg/dL	20 s 20 s	24 mg/dL: 12.1% 60 mg/dL: 8.5% 180 mg/dL: 7.1% 300 mg/dL: 2.5%	this work

*NR: not reported.

with the misaligned optical fibers exhibited almost the same sensing responses. It is noted that the refractive index (RI) of the BTB/urea solution (~1.33) was similar to the RI of the silica core of the optical fiber (~1.46). Hence, we expected that there is no significant reflection from the solution/fiber interface. Since there was no light guiding design in the proposed system, the light from the incident optical fiber served as a point light source to spread out the light into the system and the BTB/urea solution scattered the light. The receiving optical fiber then collected only part of the scattered light. When the end-end distance between the two optical fibers was far enough and the incident light was well scattered, the sensing response was insensitive to the optical fiber misalignment and the change of the end-end distance between the two optical fibers.

To verify the above mentioned mechanism, with fixed urea concentration as 180 mg/dL, we changed the end-end distance between the two optical fibers from 5 mm to 8 mm. The response at 20 s was plotted as a function of the end-end distance between the two optical fibers in Fig. S6. When the end-end distance was larger than 6 mm, changing the end-end distance did not affect the response significantly. Reducing the end-end distance to be 5 mm, however, obviously degraded the sensing response. This result supported our previous mentioned mechanism that the incident light spread into the system and the BTB/urea solution scattered the light. When the end-end distance between the two optical fibers was large enough (i.e. > 6 mm), the collecting light delivered almost the same intensity variation under urea sensing and hence the sensing response was almost unchanged. Since the tank is reusable and the test is fast, we suggest that the users establish the calibration curve when building their own reaction tank. Then, with a fixed optical fiber position, the interference due to the optical fiber end-end distance change will be eliminated.

To further assess the performance of the OFUS system compare with that of the commercial urea assay kit, synthetic urea concentrations of 24 mg/dL, 42 mg/dL, and 60 mg/dL were measured by the OFUS system (black dots) and the commercial kit (blue dots), as shown in Fig. 3(a). For the OFUS system, we put 1 μl of urea solution into the reaction tank, measure the current variation ratio (response) at the sensing time as 20 s by using 585 nm LED as light source. The urea concentration is then determined by using the calibration curve as shown in Fig. 2(f). For the commercial urea assay kit, 5 μl urea solution was mixed with the reagents. After 20 min reaction time, the saturated absorbance at 520 nm was then measured by using the micro-plate reader. A good agreement between the measurement results using the OFUS system and those using kit is obtained in Fig. 3(a), confirming that the OFUS system was ready to be used for saliva samples. For urea concentration as 24 mg/dL, 42 mg/dL, and 60 mg/dL, the RSD values were 12.1%, 15.5%, and 8.5%, respectively.

To verify the on-site salivary urea detection, we then used the OFUS system to detect saliva samples with added synthetic urea. The saliva samples were collected from healthy donors and the synthetic urea was added into the saliva samples to simulate high concentrations such as 24, 42, 60, and 180 mg/dL. Then, the samples were tested by the commercial kit to confirm that the urea concentrations are 23, 43, 68, and 171 mg/dL. According to the literatures (Cardoso et al., 2009; Peng et al., 2013; Zúñiga et al., 2012), the salivary urea concentration for CKD patients is ranged from 40 mg/dL to 270 mg/dL. Without any pretreatment of the saliva, the mixed solutions were immediately measured by using the OFUS system. The sensing response is plotted as a function of urea concentration to serve as the calibration curve using saliva samples in Fig. 3(b). The calibration curve obtained by using urea solution in Fig. 2(f) is also plotted (the blue dotted line) as a comparison. We noticed that either using saliva samples with added urea or using urea solution as the sensing target, the calibration curves are almost the same.

With the calibration curve based on saliva samples with added synthetic urea, we then started to test the saliva samples obtained from seven CKD patients. As shown in Fig. 3(c), salivary urea concentrations in the CKD patients were determined by both OFUS system and the urea assay kit. The two methods gave a quite consistent result. The correlation coefficient (r) between salivary urea concentrations obtained from the OFUS system and that obtained from the commercial urea assay kit was 0.99. This high r-value verifies that the OFUS system can be used to determine salivary urea concentration in the CKD patients.

Many previous studies had demonstrated good correlation coefficients (r = 0.75–0.99) between salivary urea and BUN concentrations

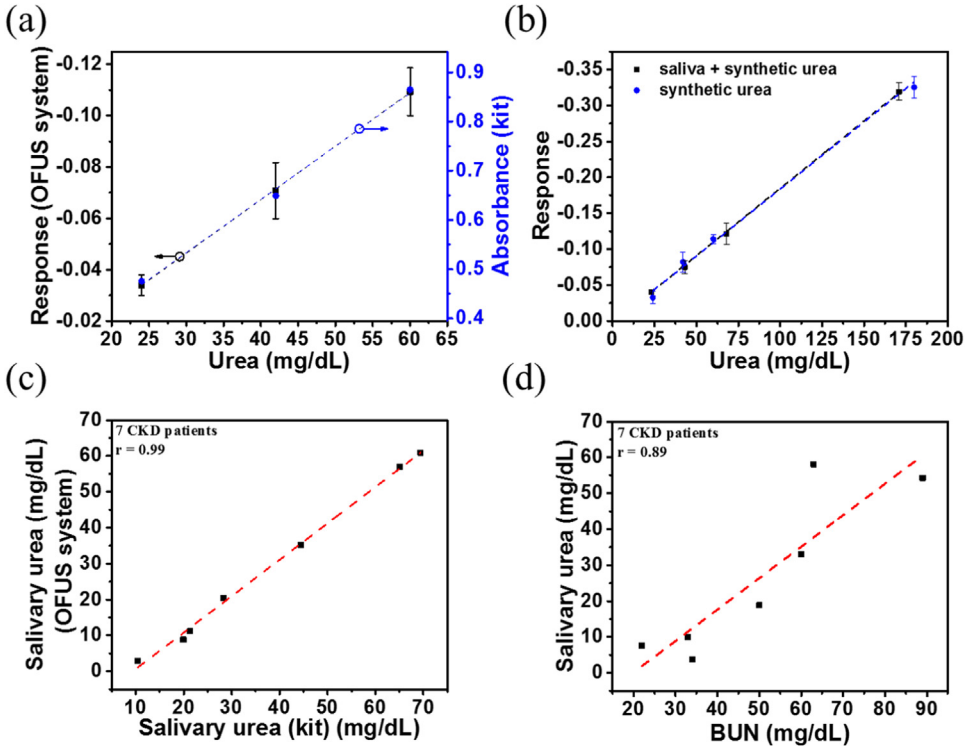

Fig. 3. (a) Synthetic urea concentrations of 24 mg/dL, 42 mg/dL, and 60 mg/dL measured by the OFUS system (black dots) and the commercial kit (blue dots). (b) The sensing response as a function of urea concentration (i.e. the calibration curve) by using saliva samples with added synthetic urea (the black dotted line). The calibration curve using synthetic urea solution in Fig. 2(f) is also plotted (the blue dotted line) as a comparison. (c) Salivary urea concentrations in the seven CKD patients obtained from the OFUS system (y-axis) and those obtained from the commercial urea assay kit (x-axis). (d) Correlation between salivary urea concentrations and BUN concentrations in the seven CKD patients in this study.

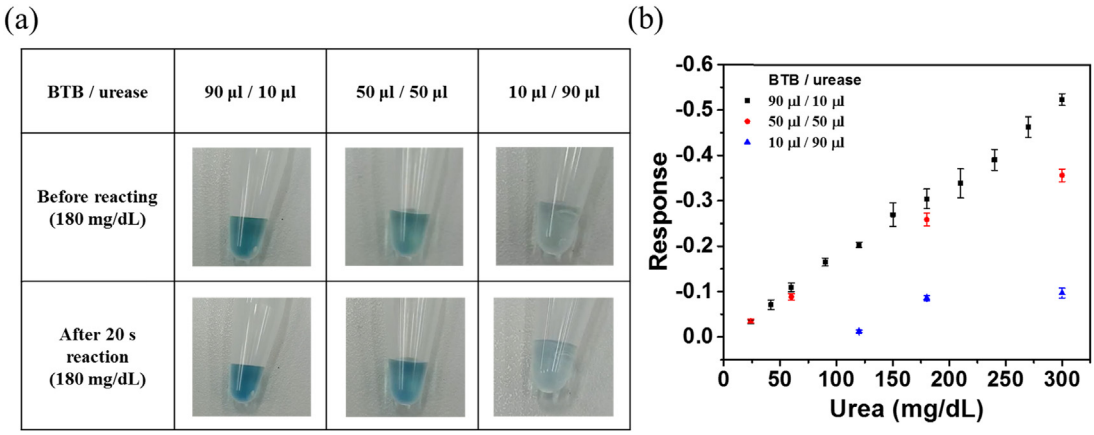

Fig. 4. (a) The photo images of the three BTB solutions before reacting and after reacting with 180 mg/dL urea. (b) The calibration curves for the three different BTB / urease solutions.

(Ahmed et al., 2015; Akai et al., 1983; Cardoso et al., 2009; Pandya et al., 2016; Sein and Arumainayagam, 1987; Tomás et al., 2008). Consistent with prior researches, our study found that salivary urea concentrations, measured by the OFUS system, was positively correlated with BUN levels, r = 0.89, shown in Fig. 3(d). This result indicates the potential future application of the OFUS system to the measurement of salivary urea concentration.

Finally, we like to demonstrate the key factors affecting the function of the OFUS system. As shown in Fig. 4(a), we adjusted the volumes of BTB and urease solutions. The total volume of the final mixed solution remained the same and the ratios of BTB/urease were 9:1, 1:1, and 1:9. The photo images of the three BTB solutions before reacting and after reacting with 180 mg/dL urea were shown in Fig. 4(a). When the ratio of BTB/urease was 9:1, the color of the solution changed from green to blue during urea detecting. When the ratio of BTB/urease was 1:9, the color of the solutions changed from light green to light blue during urea sensing. The calibration curves using the three BTB/urease solutions were shown in Fig. 4(b). When the ratio of BTB/urease was 9:1, a good calibration curve was obtained. The RSD values for urea concentrations as 24, 60, 180, and 300 mg/dL were 12.1%, 8.5%, 7.1%, and 2.5%, respectively. When BTB/urease ratio was 1:1, the responses for high urea concentration (i.e. 180 mg/dL and 300 mg/dL) were smaller than those with high BTB/urease ratio. For urea concentration as 24, 60, 180, and 300 mg/dL, the RSD values were 11.4%, 8.2%, 5.5%, and 3.9%, respectively. When BTB/urease ratio was 1:9, a very poor calibration curve was observed. For urea concentration as 120, 180, and 300 mg/dL, the RSD values were 20.1%, 6.4%, and 11.2%, respectively. The responses became very small and there was no linearity between the response and the urea concentration. We speculated that this result was due to the increased amount of BTB with a strong color change, which was reflected in the response value.

4. Conclusions

We developed a portable, low-cost, and fast salivary urea testing system (OFUS system), composed of a commercial LED, 3-D printed tank, optical fiber, and commercial photo-conductive cell to realize on-site testing with easy operation. The calibration curve of the OFUS system is stable and has high reproducibility and good repeatability. A good linear detection range of 24 – 300 mg/dL is obtained. With only 20 s detection time and only 1 µl saliva sample, the OFUS system realized on-site testing without the need to do sample pretreatment. The sensing results were confirmed by using the commercial kit. A good correlation between salivary urea and the blood urea nitrogen is also obtained. We also analyzed the influences of several interference factors such as the pH value of urea solution, the ambient light intensity, the optical fiber alignment, and the end-end distance between the two optical fibers. We verified that suppressed interference effects could be obtained by using the current variation ratio, not the current level, as the sensing response. Also, the BTB/urea solution well scattered the incident light, hence the sensing response was insensitive to the optical fiber misalignment or the change of the end-end distance between the two optical fibers.

The OFUS system had a very simple and robust design to realize on-site salivary urea detection. It exhibited RSD values comparable to other prior works. The LOD, however, was a bit high as 25.5 mg/dL. In the future, to improve the LOD, two kinds of BTB/urease solution can be prepared. One exhibits high BTB/urease concentration to increase the response in low urea concentration regime, and the other exhibits low BTB/urease concentration to suppress the signal saturation in high urea concentration regime. Then, since only 1 µl saliva sample is needed in one test, the saliva sample can be tested twice using the two solutions.

In the future, it is also expected to improve the current readout further. A high output current close to mini-amp level should be obtained by using a high-efficiency photodetector. In this way, a cheap ampere meter can be used to measure the output current. Alternatively, an electrical readout circuitry to allow wireless signal reading on a smartphone can be used to facilitate the data handling and statistical analysis. Even with current results, we expect that the simple design of the OFUS system can stimulate the clinical trials all around the world and hence to build up clear intended uses of salivary urea test.

Acknowledgments

The authors would like to thank all the master students in NCTU organic semiconductor lab for their supports for the data collection in the clinical trial. This work was supported by funding from Ministry of Science and Technology, Taiwan (Projects 108-2923-E-009-002-MY3, 107-2218-E-009-008, and 106-2221-E-009-117)

Declaration of interests

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2019.03.007.

References

Ahmed, N., Mehmood, A., Dawani, N., Roshan, S., 2015. Pak. J. Med. Dent. 4 (02), 03–07.
Ahmed, R.F., Jabber, W.F., Aldelaimi, A.A.K., 2018. J. Res. Med. Dent. Sci. 6 (2), 148–153.
Akai, T., Naka, K., Yoshikawa, C., Okamoto, T., Yamagami, S., Inoue, T., Yamada, S., 1983. Clin. Chem. 29 (10), 1825–1827.
Alev-Tuzner, B., Beyler-Cigil, A., Kahraman, M.V., Yarat, A., 2018. Int. J. Polym. Mater. Polym. Biomater. 1–10.
Badugu, R., Reece, E.A., Lakowicz, J.R., 2018. J. Biomed. Opt. 23 (5), 057005.
Bertocchi, P., Compagnone, D., 1996. Biosens. Bioelectron. 11, 1–10.
Calice-Silva, V., Vieira, M.A., Raimann, J.G., Carter, M., Callegari, J., Levin, N.W., Kotanko, P., Pecoits-Filho, R., 2014. Clin. Nephrol. 82 (6), 358–367.
Cardoso, E.M., Arregger, A.L., Tumilasci, O.R., Elbert, A., Contreras, L.N., 2009. Scand. J. Clin. Lab. Invest. 69 (3), 330–334.
Celec, P., Tóthová, Ľ., Šebeková, K., Podracká, Ľ., Boor, P., 2016. Clin. Chim. Acta 453, 28–37.
Chuang, M.Y., Chen, C.C., Zan, H.W., Meng, H.F., Lu, C.J., 2017. ACS Sens. 2, 1788–1795.
Clinton, T., Lotan, Y., 2017. Rambam Maimonides Med. J. 8 (4), e0040.
Davies, S.J., Španěl, P., Smith, D., 2014. Bioanalysis 6, 843–857.
Di Natale, C., Paolesse, R., Martinelli, E., Capuano, R., 2014. Anal. Chim. Acta 824, 1–17.
Diagnostic Salivary Progesterone Elisa Kit (FDA, CE Mark) Available at: 〈https://www.salimetrics.com/assay-kit/diagnostic-salivary-cortisol-elisa-kit/〉.
Drugs of Abuse (Collection Kit). Available at: 〈https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/DrugsofAbuseTests/ucm125761.htm〉.
Evan, R.D.R., Cooke, W., Hemmila, U., Calice-Silva, V., Raimann,, Jochen, Dreyer, G., Levin, N.W., Pecoits-Filho, R., 2018. Kidney Int. Rep. 3, 178–184.
Evans, R.D.R., Calice-Silva, V., Raimann, J.G., Hemmila, U., Craik, A., Mtekateka, M., Hamilton, F., Kawale, Z., Dobbie, H., Dreyer, G., Levin, N., Kotanko, P., Pecoits-Filho, R., 2017. Kidney Int. Rep. 2, 219–227.
Farandos, N.M., Yetisen, A.K., Monteiro, M.J., Lowe, C.R., Yun, S.H., 2015. Adv. Healthc. Mater. 4 (6), 792–810.
FDA allows marketing of first direct-to-consumer tests that provide genetic risk information for certain conditions. Available at: 〈https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm551185.htm〉.
First Rapid Home-Use HIV Kit Approved for Self-Testing. Available at: 〈https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm310545.htm〉.
Govindarajan, V., Venkatapathy, R., NirimaOza, Parameshwaran, S., Kaliamoorthy, S., Bakthavatchalam, P., Maruthamuthu, K., Pazhani, A., 2017. Int. J. Curr. Med. Pharm. Res. 3 (9), 2324–2328.
Kumar, S., Huang, J., Abbassi-Ghadi, N., Mackenzie, H.A., Veselkov, K.A., Hoare, J.M., Lovat, L.B., Španěl, P., Smith, D., Hanna, G.B., 2015. Ann. Surg. 262, 981–990.
Lopez-Giacoman, S., Madero, M., 2015. World J. Nephrol. 4 (1), 57–73.
Lopez-Ruiz, N., Curto, V.F., Erenas, M.M., Benito-Lopez, F., Diamond, D., Palma, A.J., Capitan-Vallvey, L.F., 2014. Anal. Chem. 86, 9553–9562.
Mathew, T.L., Pownraj, P., Abdulla, S., Pullithadathil, B., 2015. Diagnostics 5, 27–60.
Nagler, R.M., 2008. Clin. Chem. 54, 1415–1417.
Oncescu, V., O'Dell, D., Erickson, D., 2013. Lab Chip 13, 3232–3238.
Pallos, D., Leão, M.V., Togeiro, F.C., Alegre, L., Ricardo, L.H., Perozini, C., Ruivo, G.F., 2015. Arch. Oral. Biol. 60 (12), 1784–1788.
Pandya, D., Nagrajappa, A.K., Ravi, K.S., 2016. J. Clin. Diagn. Res. 10 (10), ZC58–ZC62.
Park, M., Hsu, C.Y., Go, A.S., Feldman, H.I., Xie, D., Zhang, X., Mifflin, T., Waikar, S.S., Sabbisetti, V.S., Bonventre, J.V., Coresh, J., Nelson, R.G., Kimmel, P.L., Kusek, J.W., Rahman, M., Schelling, J.R., Vasan, R.S., Liu, K.D., 2017. Clin. J. Am. Soc. Nephrol. 12 (5), 761–771.
Peng, C.H., Xia, Y.C., Wu, Y., Zhou, Z.F., Cheng, P., Xiao, P., 2013. Clin. Biochem. 46 (3), 275–277.
Piccione, G., Foà, A., Bertolucci, C., Caola, G., 2006. J. Circadian Rhythms 4 (1), 16.
Prasad, S., Tyagi, A.K., Aggarwal, B.B., 2016. Exp. Biol. Med. 241 (8), 783–799.
Raimann, J.G., Calice-Silva, V., Thijssen, S., Nerbass, F.B., Vieira, M.A., Dabel, P., Evans, R., Callegari, J., Carter, M., Levin, N.W., Winchester, J.F., Kotanko, P., Pecoits-Filho, R., 2016. Blood Purif. 42, 64–72.
Raimann, J.G., Kirisits, W., Gebetsroither, E., Carter, M., Callegari, J., Rosales, L., Levin, N.W., Kotanko, P., 2011. Clin. Nephrol. 76 (1), 23–28.
Renda, R., 2017. Ren. Fail. 39 (1), 452–457.
Romero, A.C., Bergamaschi, C.T., de Souza, D.N., Nogueira, F.N., 2016. PLoS One. 11 (2), e0148742.
Saalberg, Y., Wolff, M., 2016. Clin. Chim. Acta 459, 5–9.
Saran, R., Robinson, B., Abbott, K.C., Agodoa, L.Y.C., Bhave, N., Bragg-Gresham, J., Balkrishnan, R., Dietrich, X., Eckard, A., Eggers, P.W., Gaipov, A., Gillen, D., Gipson, D., Hailpern, S.M., Hall, Y.N., Han, Y., He, K., Herman, W., Heung, M., Hirth, R.A., Hutton, D., Jacobsen, S.J., Jin, Y., Kalantar-Zadeh, K., Kapke, A., Kovesdy, C.P., Lavallee, D., Leslie, J., McCullough, K., Modi, Z., Molnar, M.Z., Montez-Rath, M., Moradi, H., Morgenstern, H., Mukhopadhyay, P., Nallamothu, B., Nguyen, D.V., Norris, K.C., O'Hare, A.M., Obi, Y., Park, C., Pearson, J., Pisoni, R., Potukuchi, P.K., Rao, P., Repeck, K., Rhee, C.M., Schrager, J., Schaubel, D.E., Selewski, D.T., Shaw, S.F., Shi, J.M., Shieu, M., Sim, J.J., Soohoo, M., Steffick, D., Streja, E., Sumuda, K., Tamura, M.K., Tilea, A., Tong, L., Wang, D., Wangm, M., Woodside, K.J., Xin, X., Yin, M., You, A.S., Zhou, H., Shahinian, V., 2018. Am. J. Kidney Dis. 71 (3), S1–S672 (suppl 1)(:Svii).
Sein, K.T., Arumainayagam, G., 1987. Clin. Chem. 33 (12), 2302–2304.
Shirzaiy, M., Heidari, F., Dalirsani, Z., Dehghan, J., 2015. Diabetes Metab. Syndr.: Clin. Res. Rev. 9 (4), 332–336.
Shrivastava, A., Gupta, V.B., 2011. Chron Young Sci. 2 (1), 21.
Slowey, P.D., 2013. J. Calif. Dent. Assoc. 41 (2), 97–99 (102-5).
Soni, A., Surana, R.K., Jha, S.K., 2018. Sens. Actuators B-Chem. 269, 346–353.
Streckfus, C.F. (Ed.), 2015. Advances in Salivary Diagnostics. Springer, Berlin, Heidelberg.
Tomás, I., Marinho, J.S., Limeres, J., Santos, M.J., Araújo, L., Diz, P., 2008. Arch. Oral. Biol. 53 (6), 528–532.
Wilson, A.D., 2015. Metabolites 5, 140–163.
Won, A.J., Kim, S., Kim, Y.G., Kim, K.B., Choi, W.S., Kacew, S., Kim, K.S., Jung, J.H., Lee, B.M., Kim, S., Kim, H.S., 2016. Mol. BioSyst. 12, 133–144.
Zheng, L.Y., Umans, J.G., Yeh, F., Francesconi, K.A., Goessler, W., Silbergeld, E.K., Bandeen-Roche, K., Guallar, E., Howard, B.V., Weaver, V.M., Navas-Acien, A., 2015. Epidemiology 26 (4), 601.
Zúñiga, M.E., Estremadoyro, L.O., León, C.P., Huapaya, J.A., Cieza, J.A., 2012. J. Nephrol. 25 (3), 431.