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Research article

Electrical detection of blood cells in urine

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ABSTRACT

Available methods for detecting blood in the urine (hematuria) can be problematic since results can be influenced by many factors in patients and in the lab settings, resulting in false positive or false negative results. This necessitates the development of new, accurate and easy-access methods that save time and effort. This study demonstrates a label-free and accurate method for detecting the presence of red and white blood cells (RBCs and WBCs) in urine by measuring the changes in the dielectric properties of urine upon increasing concentrations of both cell types. The current method could detect changes in the electrical properties of fresh urine over a short time interval, making this method suitable for detecting changes that cannot be recognized by conventional methods. Correcting for these changes enabled the detection of a minimum cell concentration of 10^2 RBCs per ml which is not possible by conventional methods used in the labs except for the semi-quantitative method that can detect 50 RBCs per ml, but it is a lengthy and involved procedure, not suitable for high volume labs. This ability to detect very small amount of both types of cells makes the proposed technique an attractive tool for detecting hematuria, the presence of which is indicative of problems in the excretory system.

1. Introduction

The kidney is a vital organ in the human body with excretory, endocrine and metabolic functions [1]. Any abnormalities or damage to the kidneys can lead to serious complications and can be lethal [2]. Chronic kidney disease (CKD) is a term used to describe kidney damage that is characterized by abnormal and increased levels of albumin secretion in urine (albuminuria) or decreased kidney function that lasts for more than three months [3]. Kidney function is assessed by measuring the estimated glomerular filtration rate (eGFR), which is compared to a standardized criterion that aids in deciding the severity of CKD and thus to stratify patients. This allows for better medical interventions and treatments associated with complications, avoiding possible progression to end-stage disease.

Urine of a healthy individual should be clear, with none or very few numbers of cells [4]. Observing an increased number of cells in urine can be indicative of a serious problem that needs intervention. Hematuria or the presence of red blood cells (RBCs) in urine is an abnormal condition that might indicate serious problems in kidneys or can be a sign of progression to CKD, if not managed. Thus, accurate detection of hematuria can be of high prognostic value, in addition to being important for diagnosis.

A patient is considered to have hematuria if the number of RBCs is more than five per high-powered field (HPF) microscopy [5]. Under other criteria, even three or more cells per HPF is considered an abnormal finding, and this finding should be observed at least in two of the sediments of three collected urine samples [6]. Hematuria is described as "gross/macroscopic" when the color of blood is visible by naked eyes, or "microscopic" when the RBC numbers are few. Microscopic hematuria is only detectable by dipsticks or microscopic urinalysis. The two types of hematuria have different etiologies, but in some cases, they share common causes. In gross hematuria, the urine color can range from pink, red to brown depending upon the source of blood. Light colored blood comes

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usually from the lower urinary tract (non-glomerular), while dark colored blood is from the glomerular origin.

The two types of hematuria can be caused by various conditions, such as trauma, nephrolithiasis, postinfectious glomerulonephritis, and immunoglobulin A (IgA) nephropathy. Gross hematuria is also caused by urinary tract infections, perineal and ureteral irritations, congenital anomalies, anatomic abnormalities (e.g., tumor), acute nephritis, and coagulopathy. The microscopic hematuria, on the other hand, can occur in some diseases such as familial hematuria, sickle cell trait or sickle cell anemia, Alport syndrome nephritis and Henoch-Schönlein purpura [7]. Glomerular hematuria is more critical since it implies damage to the nephrons which filter the blood that passes through them. The leakage of RBCs into the urinary space during glomerular hematuria indicates defects in the glomerular basement membrane; these defects can result from abnormalities in the structure or composition of the basement membrane in some diseases, leading to notches and membrane rupture [8]. Similarly, inflammation or glomerulonephritis can also lead to glomerular membrane weakening and leakages a result of leukocytes infiltration into the basement membrane, in addition to the production of reactive oxygen species such as H_2O_2 [5,9] and proteinases which damage and degrade the components of the membrane, respectively [10].

Interestingly, hematuria is not only a sign of kidney damage but can itself lead to kidney damage, like hemoglobinuria-induced tubular injury [11]. The presence of metabolites released by RBCs in the lumen of nephron tubules can initiate a cascade of intracellular reactions, leading to the generation of reactive oxygen species [12], and consequently lipid peroxidation [13], caspase activation, and eventually programmed cell death (apoptosis) [14]. Taken together, hematuria can act as a diagnostic factor for the presence of kidney disease and can, at the same time, act as a prognostic marker for CKD. It is noteworthy to mention that gross hematuria is more frequent at the early stages of the disease, while microhematuria is associated with worse renal outcomes and a higher incidence of patients with end-stage renal disease (ESRD). All of this suggests that detecting microscopic hematuria is critical to predicting and recognizing patients at high risk of developing CKD, which enables healthcare professionals to take proactive measures to stop initial stages of CKD from progressing to ESRD [15, 16]. In the following section, we will discuss different methods used to asses hematuria. This will be followed by the presentation of a new approach for investigating the dielectric properties of urine samples having blood cells as a new, real-time, non-invasive method that can be of great use in the clinical setting.

2. Methods used for testing hematuria

Dipstick urinalysis is the gold standard for testing the presence of hematuria. A blood sample with hematuria generates a greenish-blue color, which results from the oxidation of a chromogenic substrate, "tetramethylbenzidine" by the peroxidase activity of hemoglobin [13]. Different companies use different chromogenic substrates for this purpose. It is believed that dipstick can detect as low as 2–5 RBC/HPF, which is equivalent to 10 RBCs per μL. However, there are some pitfalls of this method, such as false positive results in certain cases like hemoglobinuria and myoglobinuria in which there are no blood cells in urine and have different etiologies from hematuria [17, 18]. The positive results in these conditions do not result from the presence of RBCs, but rather from the pigments that are released from lysed blood cells or from muscle damage, respectively. Dipsticks can also give false positive results when bacterial peroxidases are released in urine because of infection, or because of contamination of urine with oxidizing agents used in cleaning solutions, besides menstrual blood in women. On the other hand, urine of patients taking vitamin C supplements might develop false negative results [18]. The presence of RBCs in urine can also be confirmed by a microscopic examination of the sediment of spun urine under high-powered field (HPF), or what is known as microscopic urinalysis, and the count is usually described as RBC/HPF [18]. This is the conventional method, but there is always a concern about RBC loss as a result of cells sticking to the walls of the tube during centrifugation or during decanting of the supernatant. To avoid these artifacts, some favor the counting of cells in un-centrifuged urine using a counting Coulter chamber [19]. A cytological semi-quantitative method was shown to be more accurate than both the conventional and dipstick method. In this method, the sediment of urine is spread over many slides, fixed and stained and then cells are counted under the microscope. This method was able to detect blood cells in concentrations less than 50 RBCs per ml, while the minimum concentration detected by the conventional method is 500 RBCs per ml. Surprisingly, dipsticks from different companies could detect minimum concentrations that ranged from 2×10^4 to 1×10^6 RCBs cells per ml which is much larger than the number claimed by companies [20]. Dipsticks are attractive for initial screening of urine samples since they can detect several abnormalities in urine, besides being cheap, fast and easy to use. One study has shown that using dipstick are very reliable since further microscopic analysis on same tested samples did not change the treatment decisions that were based on dipsticks results [21]. Regarding the detection of hematuria, dipsticks were found to be more accurate and consistent compared to flow cytometry and microscopic analysis when used under unstandardized condition (hyperhydration) [22].

Usually, a positive result for dipstick is followed by a microscopic examination to confirm hematuria, which consumes a considerable amount of time. A false negative result is even worse and can result in misdiagnosis which can be dangerous to patient health. More developed and sophisticated methods for urine analysis involve the use of automated analyzers. Different analyzers can have different analytical principles and might require different sample preparations. Some of them are based on flow cytometry where fresh urine samples are used to determine particle size and complexity by light scattering, and additional sample staining can give more information about DNA [23]. Others are based on image-analysis in which urine sediment is analyzed by obtaining particle images using integrated cameras which are analyzed using predefined libraries [24, 25]. Although these automated methods can save time in high-volume labs, they still require the presence of well-trained professional technicians that need to accept or reject the results or reclassify them, especially in the image-based methods. Moreover, one cannot ignore the costly charges of instruments maintenance [25]. This always is a motivation factor to find newer, cheaper methods that allow accurate and instant results, saving time besides minimizing maintenance charges. This study, thus investigated the use of electrical properties of urine to detect both RBCs and white blood cells (WBCs) in urine which can be cost-effective and save time compared to the previous methods.

2.1. Ethical approval

This research includes experimentation on human biological samples, which have been approved by United Arab Emirates University Human Ethics Committee with reference number "ERH_2017_5499". The authors confirmed that all the conducted experiments in this work were conducted according to established ethical guidelines, and informed consent obtained from the participants.

3. The current approach

Suspensions of biological cells are known to interact with an applied electric field by experiencing different levels of polarization; therefore, cells can be treated as a dielectric material whose capacitance can be affected by the electric field which can be measured by a capacitor. The non-homogenous nature of cells arises from their complex composition; cells comprise proteins, carbohydrates, nucleic acids and lipids, in addition to other minor constituents like ions and vitamins. They get polarized when subjected to the propagation of an electric field. The strength of the polarization depends on the composition and how cells interact with the suspending medium [26]. A cell suspension poses a

unique response to the electric field since it is frequency-dependent with minimally three incremental losses or dispersions known as α, β and γ dispersions [27]. Blood cells such as RBCs and WBCs suspended in the urine sample are expected to behave in a similar manner since the inclusion of biomaterials in a biological fluid is known to alter its dielectric properties. Thus, this study investigated the effects of increasing concentrations of RBCs or WBCs in urine on the capacitance measurements of urine. A noteworthy feature of this method is that there are no major pre-processing steps. Detection of changes in the electrical properties that correlate well with concentration can be of importance since it will enable the extraction of cell concentration, a parameter that is of high diagnostic and prognostic value, especially in the case of hematuria where high sensitivity is required to detect a small number of cells. Figure 1a depicts a biological cell with the negatively-charged glycocalyx (a carbohydrate coating) covering its cell surface. The glycocalyx attracts the positively-charged free ions in the suspending medium.

Figure 1b illustrates how cells suspended in urine are randomly distributed inside a capacitor. When a cell is placed under the effect of an applied electric field, it gets polarized and one type of polarization results from the diffusion of the charged ions present at the cell surface, resulting in a big dipole (see Figure 1c). For simplicity and modeling, the cell distribution inside a capacitive structure is assumed as two separate zones, and a parallel model is shown in Figure 1d. This assumption is based on our earlier work which indicated that when cells are added to control media, the capacitance increases [26]. With more cells, higher capacitance values are observed. The volumes of the cells and medium zones are the same as their respective actual volumes in the suspension in both parallel and series representations.

4. Materials and methods

4.1. Collection of urine samples

A fresh, early morning urine (EMU) sample (30–50 ml) was collected in a sterile urine container with no additional preservatives.

4.2. Separation of RBCS and WBCS

Whole blood (5–10 ml) collected in an EDTA vacutainer was centrifuged at 377x*g* for 10 min at 4 °C. Blood separates into three components: plasma (top), buffy coat (middle) and RBCs (bottom). The top plasma layer was carefully removed and discarded without disturbing the buffy coat. The buffy coat containing the WBC was carefully transferred to a fresh 50-mL falcon tube using a Pasteur pipette. To get rid of the contaminating RBCs in the buffy coat fraction, 10 mL of RBC lysis buffer (2 mM Tris HCl, 5 mM $MgCl_2$, pH 7.5) was added to the buffy coat and mixed, so that the RBCs were lysed while maintaining the WBCs intact. The WBCs were pelleted at 672x *g* for 20 min at 4 °C. RBC lysis step was carried out twice to completely remove the RBCs. The WBCs were then suspended in 10 mL of Dulbecco's Modified Eagles Medium (DMEM, HyClone) and counted using a hemocytometer to prepare the required dilutions. Meanwhile, the bottom RBC-containing layer was transferred to a fresh 50-mL falcon tube and washed twice with 5 mL of PBS, suspended in 10 mL of DMEM, and counted using a hemocytometer to prepare the required dilutions.

4.3. Preparation of dilutions

The purified RBC- and WBC-cell suspensions were prepared separately using fresh urine. The dilutions initially prepared were 10^6, 10^4, 10^2, 10^1 (cells/ml) in DMEM for RBCs and WBCs separately. Just before loading into the co-axial adaptor for electrical characterization, the cell suspension in DMEM was centrifuged at 1,050x*g* for 1 min. The supernatant was removed carefully and resuspended in 1 ml of the fresh urine. The sample was then used for electrical characterization. After the electrical characterization, the same sample was collected in a microcentrifuge tube and centrifuged at 1,050×*g* for 1 min. The supernatant was carefully removed and loaded into the coaxial cable for electrical characterization.

4.4. Electrical measurements

The electric measurements were conducted by loading 500 μl of each cell suspension in urine or urine alone inside an open-ended coaxial cable connected to Gamry 3000 equipment (USA). The instrument can measure current from 3 amps to 300 pico-amps over a range of frequency from 100 MHz to 100 KHz. The coaxial adaptor is composed of inner and outer conductors with dimensions of 2 and 5 mm, respectively, and a length of 7 mm. The control media and various cell suspensions were loaded into the coaxial adaptor. The instrument can record different types of electrical measurements such as capacitance-voltage profiles. Due to the frequency and current ranges used, the device can measure a capacitance of high precision (up to zepto-Farad). Figure 2 illustrates the electrical modeling and an electric circuit for blood cells in urine. Figure 2c represents the actual experimental setup employed.

4.5. Measurements protocol

The experiments measuring the electrical properties of cells in urine were conducted as follows:

1 The control sample represents fresh urine. That is the zero-cell concentration urine sample for which the capacitance was recorded.
2 Next, either red or white blood cells were suspended in urine with the maximum concentration and the capacitance was recorded.
3 Next, serial suspensions were made from the concentrated cell sample using fresh urine
4 The suspensions were measured from high to low concentration.
5 The urine dynamic behavior, i.e., change over time was de-embedded from the values of the cell suspensions in urine.
6 Since the urine dynamic behavior depends on many parameters, such as the food, drink or human activities, therefore, two fresh urine were used on different days.

5. Results

Figure 3a displays curves for capacitance values measured over a range of frequencies (1–10^5 Hz) for urine samples spiked with increasing concentrations of RBCs (10^2 to 10^6 cells/ml). The electrical measurements were made by loading the cell suspensions made in urine into the coaxial adaptor of the Gamry instrument followed by recording their electrical potential. In general, capacitance curves displayed a smooth pattern with the highest values existing at low frequencies. A gradual decrease in capacitance values occurred as the frequency applied in the capacitor increased. The capacitance-frequency curve for urine alone (sample P0 in Figure 3a), which acted as a control sample, was observed to be below the rest of the curves, indicating a low capacitance potential of urine. Unexpectedly, the capacitance values for urine/blood suspensions were inversely proportional to the concentration of RBCs; the sample with the highest number of cells (10^6) generated the lowest curve, while curves were found to shift to higher values with decreasing concentrations of blood cells (Figure 3a). Figure 3b displays curves of suspensions where the urine samples were spiked with increasing concentrations of WBC. Similar to the pattern observed with RBCs, urine samples spiked with the highest concentrations of WBCs resulted in the least value of capacitance and thus capacitance was inversely proportional to the concentration of cells in urine. Figure 3c and d display changes in capacitance values of the fresh urine sample over time that was used to make the cell suspensions. This was done by repeatedly measuring urine capacitance values at time points at which each suspension of RBC or WBC was made. In general, the capacitance values for urine samples in both sets of experiments displayed a stable increase with

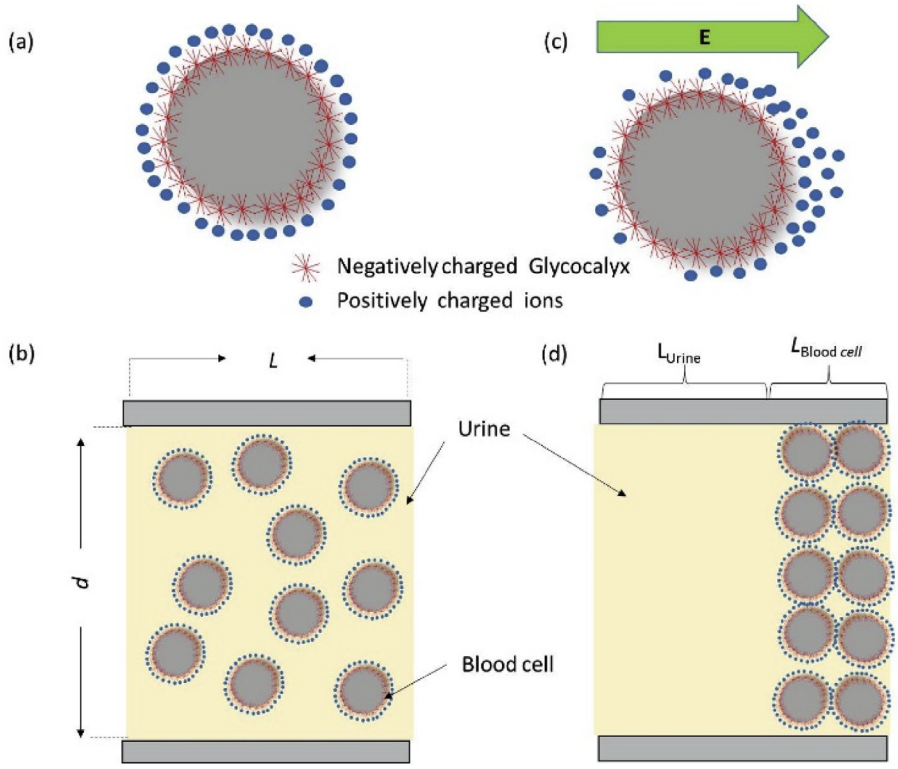

Figure 1. (a) Diagram of a blood cell with a negatively charged glycocalyx that is coated by positively charged ions in urine. (b) Illustration of the random distribution of blood cells in a suspension medium (urine). L is the length of the total capacitor; d is the distance between the two electrodes of the capacitor. (c) A diagram of how the surface-bound charged ions diffuse under the effect of an applied electric field, leading to cell polarization. (d) An alternative two-zone parallel capacitive model is presented of cell distribution inside a capacitive structure. L_{Urine} and $L_{Blood\ cell}$ are lengths of capacitors zones occupied by the medium (urine) and blood cells, respectively.

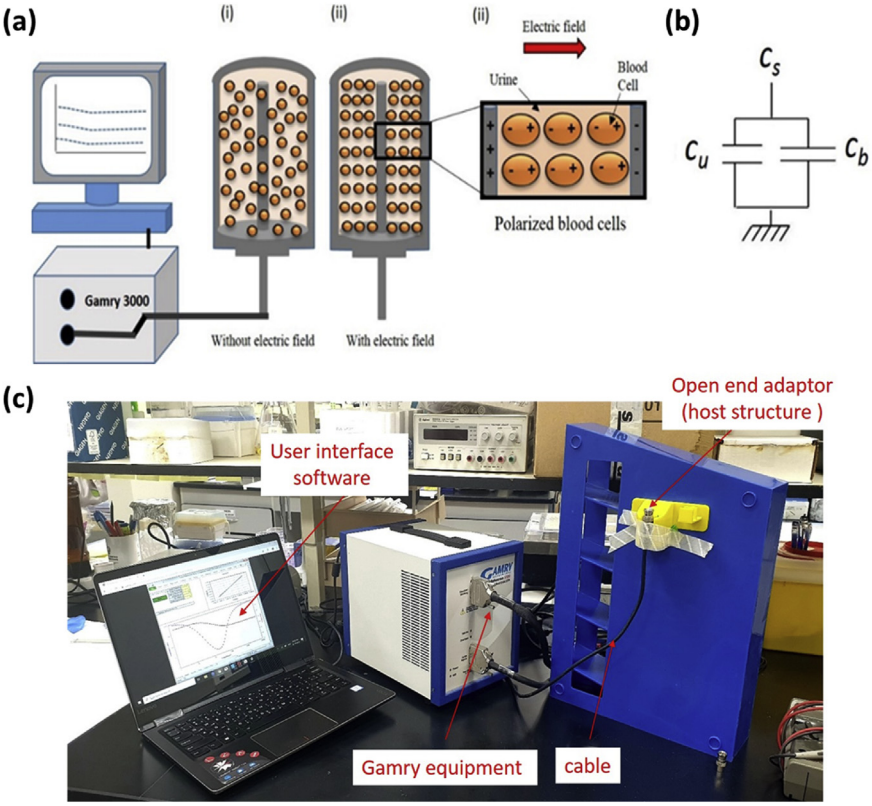

Figure 2. Illustration of the electrical modeling of blood cells suspended in urine. (a) panel (i) demonstrates the random distribution of blood cells before the application of DC bias, panel (ii) shows the distribution of blood cells after the application of DC bias, and panel and (iii) explains how cells act as dipoles when exposed to an electrical field. (b) The depiction of the corresponding equivalent circuit model, where C_S is the suspension (blood cells and urine) capacitance; C_U, is the urine capacitance; and C_b is the capacitance of the blood cells. (c) Actual photo of the experimental setup utilized in this study to measure the capacitances of various cell suspensions.

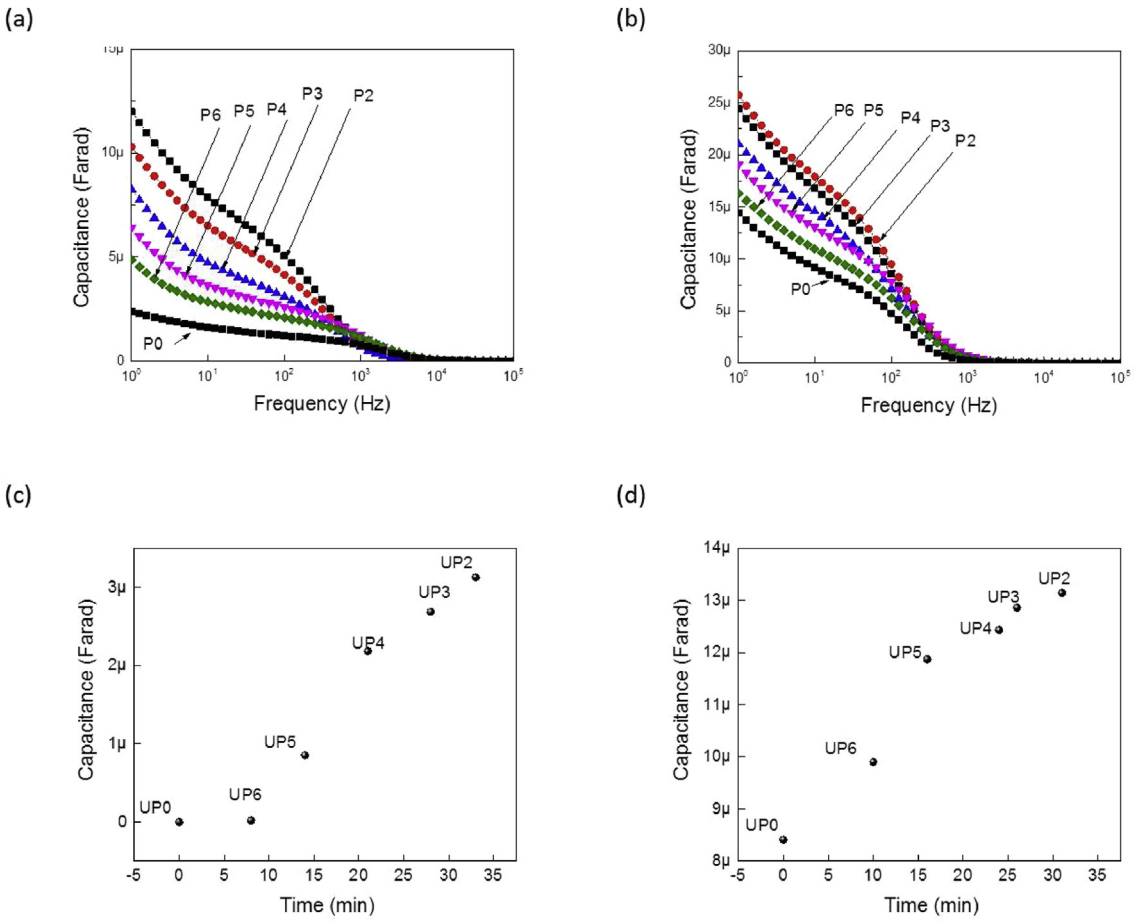

Figure 3. Dielectric properties of urine and cells suspensions measured over a range of frequencies. (a) Capacitance versus frequency profile for increasing concentrations of red blood cells (RBCs) and (b) white blood cells (WBCs) in urine. Curves labeled with P0, P2, P3, P4, P5, and P6 have concertation of 0, 10^2, 10^3, 10^4,10^5 and 10^6 cells/ml respectively. (c) Time-dependent changes in fresh urine sample capacitance alone devoid of cells that were used for RBC and (d), WBC suspensions. The changes in capacitance versus time have been measured at 1Hz. UP0, UP2, UP3, UP, UP5, and UP6 are urine samples measured electrically at the times shown in the figures and were exactly the time at which different suspensions were freshly mixed and measured.

time (measured over 35 min) as shown in Figure 3c and d, revealing that urine (the control sample) alone undergoes dynamic changes that can affect its electrical parameters, leading to a gradual increase in capacitance with time.

Figure 4a displays curves for capacitance values measured over a range of voltages (-0.5 to +0.5V) for urine samples containing increasing concentrations of RBCs. As with the capacitance-frequency profile, the capacitance-voltage curve for a urine sample (as a control) was observed at the bottom of all curves, showing less capacitance potential for urine while the capacitance values for the rest of the samples were inversely proportional to the concentration of cells in the samples.

A similar pattern was observed for urine samples containing increasing concentrations of WBCs (Figure 4b) as seen in Figure 3b. To determine if the inverse relation being observed with increasing cell concentrations was due to the instability observed with urine alone, the capacitance-voltage values for cell suspensions being observed were corrected for those of urine alone. Figure 4c and d show corrected values of capacitance-voltage curves displayed in Figure 4a and b, respectively, after de-embedding the capacitance value of the control urine sample from its suspension capacitance values. Briefly, each cell suspension was reconstituted just before the measurement was performed. After the measurement, the sample was centrifuged and the supernatant representing the fresh urine status at this time was subjected to electrical measurements. The capacitance values of each supernatant urine sample were deducted from its corresponding cell suspension. By performing this step, the capacitance values were corrected for any artifacts that might have arisen from any possible changes taking place in urine over time. Plotting the curves after the correction step revealed a more logical pattern where spiking more cells into urine led to a stepwise increase in the capacitance potential of urine. This data also revealed that 100 cells/ml could easily be distinguished from urine alone for both cell types across most of the range of frequency tested (Figure 4c and d).

Next, we compared the limit of detection of our electrical technique with that of the current method of choice for hematuria, the "urine dipstick" method. Towards this end, the red blood cell suspension in urine having the highest concentration of cells at 10^6 RBCs/ml was tested by the dipstick. Surprisingly, the dipstick revealed the normal status of urine with no indication for of the presence of red blood cells although dipsticks are claimed to be sensitive with the ability to detect from 2×10^4 to 1×10^6 cells/ml, as mentioned in Figure 5. On the other hand, our method could detect the concentration of RBCs with nearly 10,000-fold less number of cells/ml (as low as 100 cells/ml).

Next, the capacitance values in Figure 4c and d were used to extract the dielectric constants for cell suspensions over a range of voltages, and those were normalized by calculating the ratio between each dielectric constant of each suspension and the dielectric constant of urine control sample measured at the very beginning of the measurement. The normalized values of the dielectric constants were plotted for both types of cell suspensions, as shown in Figure 6a and b. Calibration curves were generated by plotting these ratios calculated at -0.25V (the mid of the

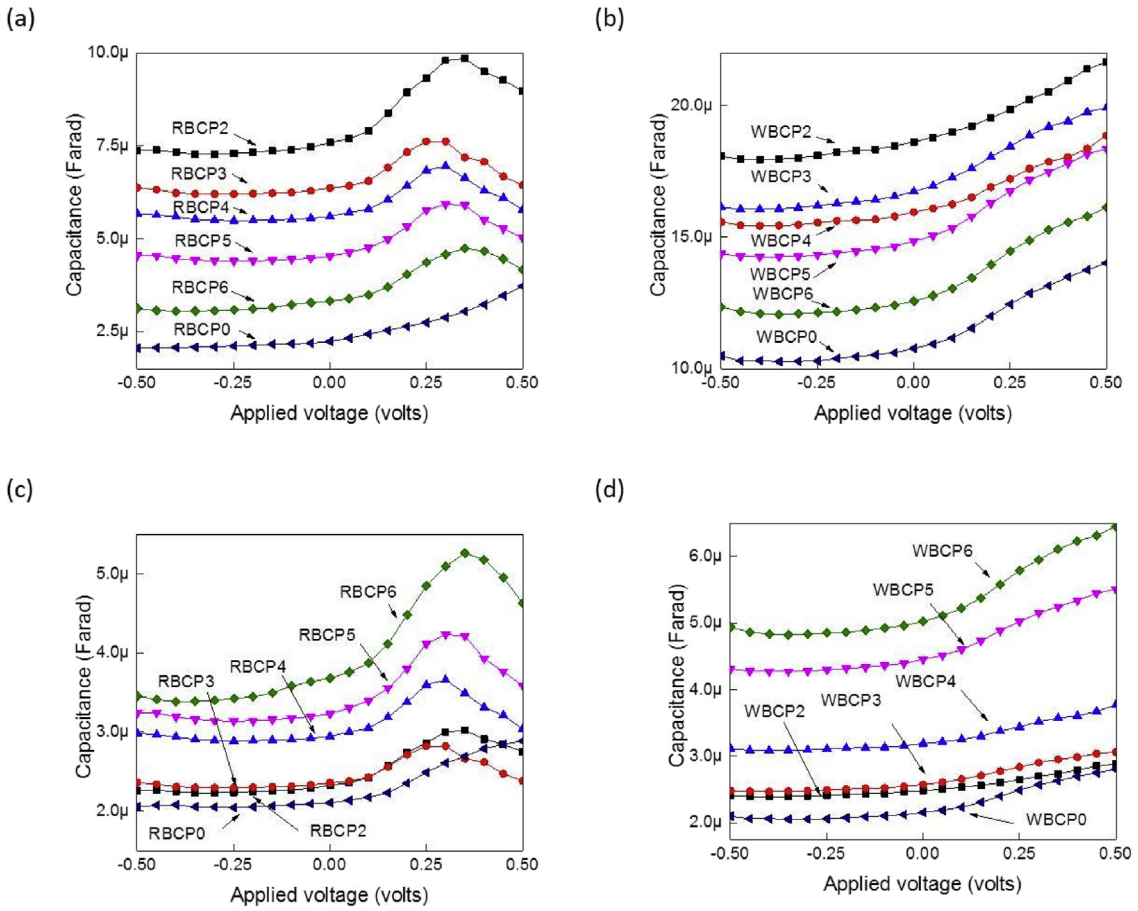

Figure 4. Capacitance-voltage (CV) profiles of urine samples containing increasing concentrations of RBCs and WBCs. (a) Capacitance-voltage profile of control urine sample and urine samples spiked with increasing concentrations of red blood cells (RBCs). Samples RBCP0, RBCP2, RBCP3, RBCP4, RBC5 and RBC6 had the following cell concentrations: 0, 10^2, 10^3, 10^4,10^5 and 10^6 cells/ml, respectively. (b) Capacitance-voltage profile of urine and urine samples with increasing concentrations of white blood cells (WBCs). Samples WBCP0, WBCP2, WBCP3, WBCP4, WBC5 and WBC6, had the following cell concentrations: 0, 10^2, 10^3, 10^4,10^5 and 10^6 cells/ml, respectively. (c) The CV values of each suspension were re-calculated by de-embedding the capacitance value of urine "alone" obtained by centrifuging and measuring the supernatant capacitance after each suspension capacitance measurement from the capacitance value of the suspension of RBCs (d). Similarly, re-calculation of WBC suspension CV values as in (c) after de-embedding the values of the urine alone.

negative voltage range) for each concentration of cells as seen in Figure 6c. Figure 6c represents the change in dielectric constant of the suspension versus the concentration of both cells type. Meanwhile Figure 6d was used to determine the number of cells in urine by extracting the corresponding dielectric constant from the measured suspension capacitance. With high cell concentration, Figure 6d revealed the possibility of identifying the cell type if the value of the extracted normalized constant was higher than 1.75 (as indicated by the vertical line). For concentrations with less than 10^4 cells per ml, both RBCs and WBCs exhibited approximately the same trend, making it difficult to use these calibration curves for identification below this range. Nevertheless, as can be concluded from the data displayed in Figure 4c and d, the recorded capacitances per the two types of cells with the same concentration values were different. Hence, the capacitance per single cell can be used for differentiation between the cell types.

6. Discussion

In this study, we have characterized the dielectric properties of urine followed by the ability to detect, quantitate, and distinguish between two different types of blood cells present in urine based on their electrical parameters. Our results show that using our approach, we could detect at a minimum of 10^2 blood cells/ml of urine. Studying the dielectric properties of urine is gaining more interest in the last few years. Such studies can pave the way for fast and non-invasive methods that can be very practical for clinical utilities. For example, in one study, electrical measurements were recorded for urine samples of normal and CKD patients with proteinuria at frequencies ranging from 0.2GHz to 50GHz and at three different temperatures [28]. Below 7 GHz, the urine of CKD patients had higher dielectric properties than normal subjects with differences becoming more significant at higher temperatures of 30 °C and 37 °C. This trend was reversed beyond 7GHz. Similarly, the positive correlation between proteinuria levels and dielectric properties was observed below 7GHz, while negative correlation was observed above this point. The lower relaxation frequency observed in samples with proteinuria was explained by a decrease in bulk water leading to slower relaxation times [28]. Similar results were also shown when urine of diabetic and CKD patients was compared to normal subjects, but differences were more significant between normal and CKD patients [29]. Other studies have investigated the effect glycosuria on the dielectric properties of urine and observed an increase in dielectric constant with increasing glucose in urea [30, 31, 32, 33]. All these studies demonstrate biomaterial dependency of urine dielectric properties. To our knowledge, no studies on the dielectric properties of urea from patients exhibiting hematuria have been conducted, nor on urine taken from patients with urinary tract infections. As discussed, the presence of RBCs is very important as a prognostic factor for CKD, while WBCs can indicate the presence of infection.

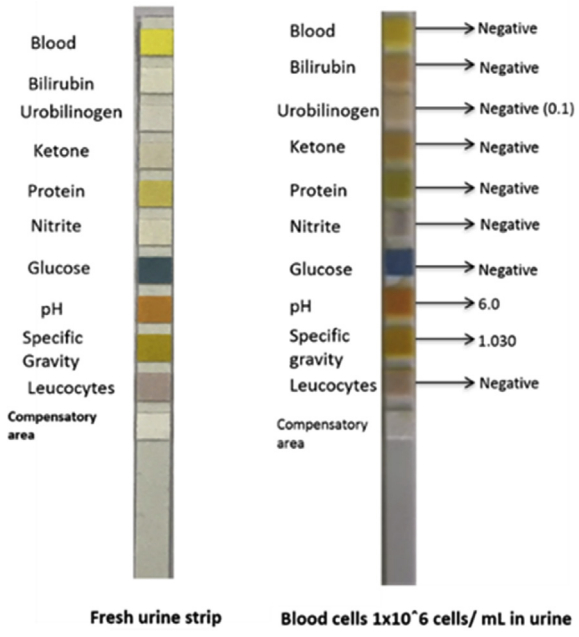

Figure 5. Dipstick analysis of fresh urine containing one million cells/ml. The strip on the left shows the urine strip dipped in fresh urine without any cells, while the strip on the right shows the strip with 10^6 cells/ml.

In the present study, we did not use real samples from patients; rather, to establish the "proof-of-principle", we investigated the effect of RBCs and WBCs on urine dielectric properties. This was achieved by spiking fresh urine with increasing concentrations of RBCs and WBCs. Surprisingly, the dielectric properties were not to our expectations since there was an inverse correlation between the urine capacitance-frequency curves and the concentration of cells (Figure 3a and b). This unexpected result raised the question of the stability of urine which possibly affected its electrical properties, leading to this artifact. To test this possibility, the dielectric property of the fresh urine sample was measured after each suspension reading, by spinning the suspension and removing the cells. Interestingly, we could observe a stepwise increase in the capacitance-frequency (CF) profile of urine alone with time (Figure 3c and d). This finding highlights the issue of urine stability over time. These observed dynamic changes in urine are possibly ascribed to some chemical reactions that can take place, altering the chemical composition of urine and consequently altering the intrinsic capacitance of urine over time. As a biological fluid, urine contains high levels of sodium chloride, phosphate, potassium, urea and trace levels of calcium, sulfate, and magnesium [34], and fresh urine pH ranges from 5.6 to 6.8 [35]. Urea is one of the components that is degraded by the action of urease enzyme found in some infectious microorganism, breaking it into ammonia and carbon dioxide, thus raising the pH of urine up to 9 [36]. One study has shown that changes in the pH and ammonia concentration of fresh urine reach a steady state after 60–72 h [33]. Moreover, this change is accelerated at higher temperatures (e.g., 25 °C and 30 °C) [37]. In another study, no significant changes in urine sample parameters were observed after 1 h, but some parameters changed after a 24-hour period of refrigeration [38].

In this study, we show that there are dynamic changes in urine that take place in short periods of times (30 min) and that these changes can be detected electrically, but not by conventional methods. It is known that electrode polarization (electric double layer) is augmented by the presence of free mobile ions in a suspension and this phenomenon can lead to an increase in capacitance values of a suspension [39]. We suggest that the dynamic changes of urine with the generation of free ammonia ions and carbonate ions may lead to this increase in capacitance which was observed over time. Another explanation can be an increase in the density of dipoles as a result of dynamic chemical changes. In fact, this was proposed to explain the high dielectric constant observed in the blood of diabetic people [40]. Furthermore, our results reveal that the capacitance-voltage (CV) profiles of the cell suspensions in urine (Figure 4a and b) had a pattern similar to the one observed in capacitance-frequency profiles. We can describe the capacitance of cells suspensions by the following formula:

$$C_{\text{suspension}} = C_{\text{urine}} + C_{\text{cells (RBC or WBC)}}$$

This formula shows that by de-embedding CV values of each urine control sample from the CV values of the urine suspensions made at the corresponding times should yield the actual CV value of cells (Figure 4c and d). As observed, after the de-embedding process, the CV curves became positively correlated with cell concentrations in urine. The ratio of the dielectric constant of each cell concentration (RBC/WBC) to the dielectric constant of fresh urine was calculated for each value over the range of voltages, as shown in Figure 6a and b. The ratios calculated at (-0.25V) were plotted against each cell concentration to get ($\varepsilon_{RBC/WBC}/\varepsilon_{urine}$) concentration curves (Figure 6c). These curves were used to extract the concentration of cells in urine. It is very important to note that this technique can detect cells numbers as low as 100 cells per ml that is even more sense than any available urine dipstick test since they can detect a minimum of 2×10^4 to 1×10^6 RBCs cells per ml. Similarly, WBCs could be detected in low concentrations as well at 100 cells/ml. We would like to stress that the ability to detect the dynamic changes in urine sample alone can also be important, especially if they result from a bacterial activity that can result in even a higher rate of change in infected urine. This can be faster than the conventional microbiological techniques that require lengthy microbial culture and proper interpretations. In our case, a high ratio of the rate of change of a suspected sample to the rate of a normal sample could act as an indication of infection since the urine sample was not handled in a sterile manner once used for the dilutions. Further investigation of this novel method and validating it can introduce easier and more efficient modalities for urine examination, especially in high volume labs where time is critical.

It is well-known that cell membranes are composed of proteins and glycoproteins embedded in a fluid lipid bilayer [41]. The Sialylated glycoproteins of the RBC membrane are responsible for a negatively charged surface. This creates a repulsive electric zeta potential between cells which helps in preventing any interactions between RBCs and the other cells, especially between each other. On the other hand, urine is an aqueous solution composed of urea, chloride, sodium, potassium, creatinine and other dissolved ions, and inorganic and organic compounds [42]. Bo et al. have considered an ionic solutions time-variant dielectric constant profile with change in ion positions due to the Brownian dynamics [43]. It is proven that the motion of ions in aqueous solution is driven by interactions among ions [44, 45]. Therefore, in this study, for the sake of simplicity, we assumed that the ionic interactions and concentration was the major contributor to the dielectric constant changes of urine with time, and there were no other interactions between the cells and the urine constituents.

In future, we would like to use "microfluidics" to improve the ability to distinguish between different cell types. Microfluidics allows electrical characterization at the single cell level, providing higher accuracy of results and thus better differentiation between cells. The composition of biotic materials and suspensions on a unit level will help in better understanding their biochemical and physiological states. This should help in the exact identification of the type of cells present in the suspension. The basic setup of a microfluidic channel is depicted in Figure 7, which incorporates a host where the sample is loaded. In our case, the sample could be urine with red and white blood cells. This system contains a pump which regulates the pressure and flow of the sample into the microfluidic chamber; the chamber contains sensing electrodes for the analysis of the loaded cell suspension. The electrical conditions for investigation can be applied with the help of the sensing electrodes. The

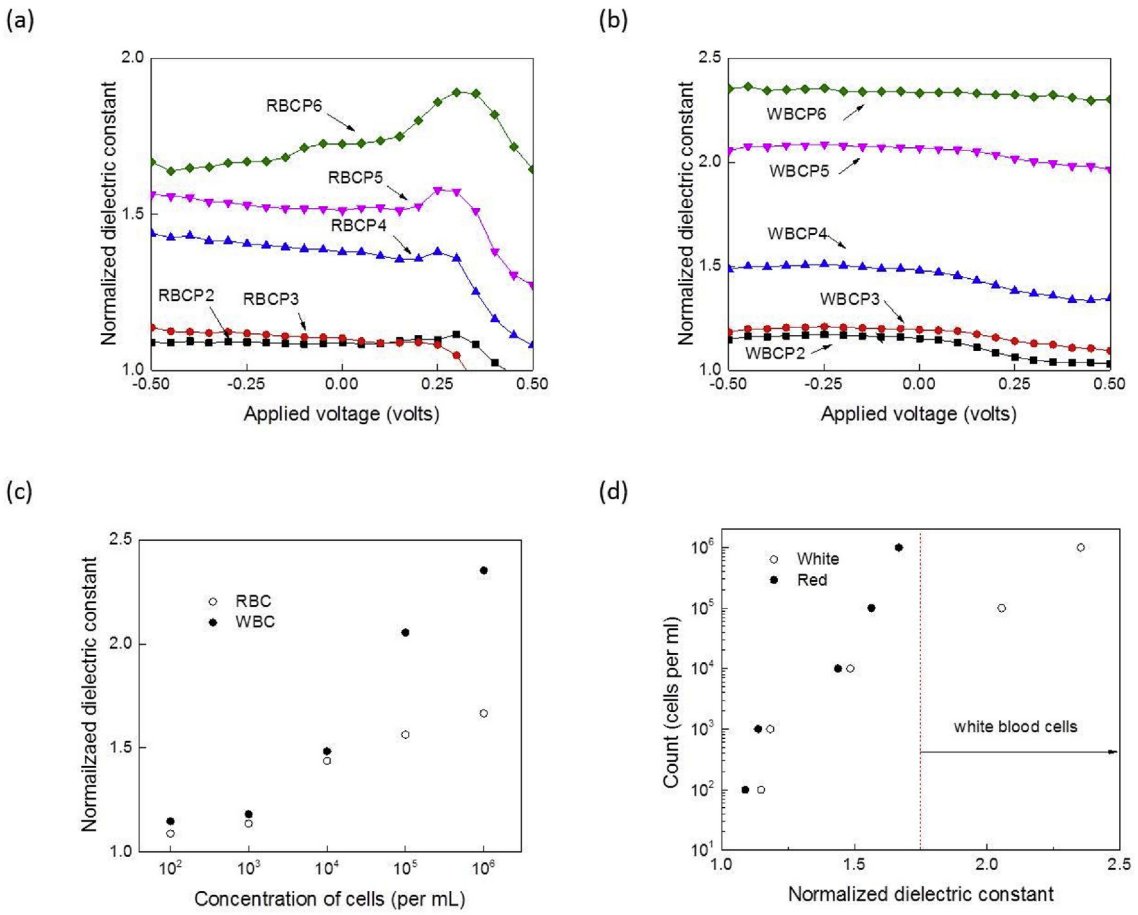

Figure 6. Extraction of the dielectric constants and cell counts from the electrical measurements. (a) Ratios of the dielectric constant of each cell suspension to the dielectric constant of urine sample plotted over a range of voltages (-0.5 to +0.5 V) for red blood cells (RBCs). Samples RBCP0, RBCP2, RBCP3, RBCP4, RPC5 and RPC6 had 0, 10^2, 10^3, 10^4,10^5 and 10^6 cells/ml, respectively. (b) Same calculations were performed for the white blood cell (WBC) urine suspensions. Samples WBCP0, WBCP2, WBCP3, WBCP4, WBC5 and WBC6 contained 0, 10^2, 10^3, 10^4,10^5 and 10^6 cells/ml, respectively. (c) Ratios calculated at a voltage of -0.25V were plotted against cell concentration for RBCs and WBCs. (d) Re-plotting figure (c) with reversed axes to calculate cell counts per ml based on electrical parameters.

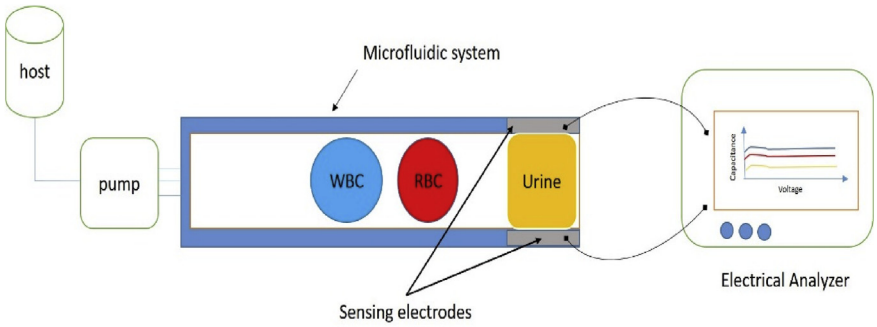

Figure 7. Proposed single cell detection and identification.

microfluidic-based system incorporates an electrical analyzer along with dedicated software which will help to apply specific electrical conditions and collect the corresponding measurements. These set of measurements are then processed and analyzed to detect and identify any existing cells in the suspension.

7. Conclusion

A new and easy method for the detection of cells in urine is proposed in this study. The method involves label-free identification and quantification of cells in urine based on electrical parameters without any pre-processing steps. This method is promising since substances such as viruses or even whole cells begin to appear in urine before people are aware that they might have a problem, and accurate detection is important before the condition becomes worse. This method is based on the use of capacitance-voltage measurements to detect the presence of blood cells suspended in urine. Any deviation from the normal composition of urine can be detected due to the changes in the electrical parameters of the urine when subjected to an electric field. This method could detect dynamic changes that took place across time and affect the electrical properties of urine even in the absence of cells. Such changes can mask the electrical changes that can be imposed by the abnormal

presence of cells. By including a de-embedding step, the masking effect was removed, and the electrical changes became more relevant and correlated well with the increasing number of blood cells. The de-embedding step was also an essential step to eliminate the effect of fluctuating and varying chemistry of the urine that exists in the same patient throughout the day or even among different individuals. This method can also be potentially used for identifying the type of cells present by comparing the capacitance measurements, which could provide a valuable tool for discriminating different urine samples of different composition. Knowing the individual electrical response of each cell component can help in explaining the effect that will be observed when they are combined. It is a highly sensitive, safe, low-cost and reliable method with minimal sample processing. It can be considered as a point-of-care test as the results can be shared instantaneously with the members of the medical team.

Declarations

Author contribution statement

M. Al Ahmad, N. Nasir, S. Raji, F. Mustafa, T. Rizvi, Z. Al Natour and A. Naqbi Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] M. Sahay, S. Kalra, T. Bandgar, Renal endocrinology: the new frontier, Ind. J. Endocrinol. Metab. 16 (12) (2012) 154–155.
[2] R. Thomas, A. Kanso, J.R. Sedor, Chronic kidney disease and its complications, Prim. Care 35 (2) (2008) 329–344.
[3] KDOQI; National Kidney Foundation, KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease, Am. J. Kidney Dis. 47 (5 Suppl 3) (2006) S11–145.
[4] J. Coresh, B.C. Astor, T. Greene, G. Eknoyan, A.S. Levey, Prevalence of chronic kidney disease and decreased kidney functioning the adult US population: third National Health and Nutrition Examination Survey, Am. J. Kidney Dis. 41 (1) (2003) 1–12.
[5] L.G. Feld, W.R. Waz, L.M. Pérez, D.B. Joseph, Hematuria. An integrated medical and surgical approach, Pediatr. Clin. N. Am. 44 (5) (1997) 1191–1210.
[6] G.D. Grossfeld, J.S. Wolf, M.S. Litwin, H. Hricak, C. Shuler, D.C. Agerter, P.R. Carroll, Asymptomatic microscopic hematuria in adults: summary of the AUA best practice policy recommendations, Am. Fam. Physician 63 (6) (2001) 1145–1154.
[7] K.H. Pade, D.R. Liu, An evidence-based approach to the management of hematuria in children in the emergency department, Pediatr. Emerg. Med. Pract. 11 (9) (2014) 1–13.
[8] S. M Jalalah, I.H. Alzahrani, P.N. Furness, Glomerular changes in microscopic haematuria, studied by quantitative immunoelectron microscopy and *in situ* zymography, Nephrol. Dial. Transplant. 17 (9) (2002) 1586–1593.
[9] R.J. Johnson, S.J. Guggenheim, S.J. Klebanoff, R.F. Ochi, A. Wass, P. Baker, M. Schulze, W.G. Couser, Morphologic correlates of glomerular oxidant injury induced by the myeloperoxidase-hydrogen peroxide-halide system of the neutrophil, Lab. Investig. 58 (3) (1988) 294–301.
[10] M. C Vissers, C.C. Winterbourn, Gelatinase contributes to the degradation of glomerular basement membrane collagen by human neutrophils Collagen, Collagen Relat. Res. 8 (2) (1988) 113–122.
[11] M.J. Tracz, J. Alam, K.A. Nath, Physiology and pathophysiology of heme: implications for kidney disease, J. Am. Soc. Nephrol. 18 (2007) 414–420.
[12] J.M. Rifkind, E. Nagababu, Hemoglobin redox reactions and red blood cell aging, Antioxidants Redox Signal. 18 (17) (2013) 2274–2283.
[13] Y.I. Miller, S.M. Altamentova, N. Shaklai, Oxidation of low-density lipoprotein by hemoglobin stems from a heme-initiated globin radical: antioxidant role of haptoglobin, Biochemistry 36 (40) (1997) 12189–12198.
[14] E. Homsi, P. Janino, J.B. de Faria, Role of caspases on cell death, inflammation, and cell cycle in glycerol-induced acute renal failure, Kidney Int. 69 (8) (2006) 1385–1392.
[15] T. Shima, Hematuria is a risk factor towards end-stage renal disease - a propensity score analysis, Nephrol. Renal Dis. 2017 (2017).
[16] J.A. Moreno, C. Martín-Cleary, E. Gutiérrez, A. Rubio-Navarro, A. Ortiz, M. Praga, J. Egido, Haematuria: the forgotten CKD factor? Nephrol. Dial. Transplant. 27 (1) (2012) 28–34.
[17] K.D. Phadke, M. Vijayakumar, J. Sharma, A. Iyengar, Consensus statement on evaluation of hematuria, Indian Pediatr. 43 (11) (2006) 965–973.
[18] P. Veerreddy, Hemoglobinuria misidentified as hematuria: a review of discolored urine and paroxysmal nocturnal hemoglobinuria, Clin. Med. Insights Blood Disord. 6 (2013) 7–17.
[19] P. Kincaid-Smith, K. Fairley, The investigation of hematuria, Semin. Nephrol. 25 (3) (2005) 127–135.
[20] S.C. Freni, G.J. Heederck, C. Hol, Centrifugation techniques and reagent strips in assessment of microhematuria, J. Clin. Pathol. 30 (4) (1977) 336–340.
[21] W.W. Jou, R.D. Powers, Utility of dipstick urinalysis as a guide to management of adults with suspected infection or hematuria, South. Med. J. 91 (3) (1998) 266–269.
[22] A. Bataille, M. Wetzstein, A. Hertig, S. Vimont, E. Rondeau, P. Galichon, Evidence of dipstick superiority over urine microscopy analysis for detection of hematuria, BMC Res. Notes 9 (1) (2016) 435.
[23] V. Khejonnit, B. Pratumvinit, K. Reesukumal, S. Meepanya, C. Pattanavin, P. Wongkrajang, Optimal criteria for microscopic review of urinalysis following use of automated urine analyzer, Clin. Chim. Acta 439 (2015) 1–4.
[24] Z. Zaman, G.B. Fogazzi, G. Garigali, M.D. Croci, G. Bayer, T. Kránicz, Urine sediment analysis: analytical and diagnostic performance of sediMAX - a new automated microscopy image-based urine sediment analyser, Clin. Chim. Acta 411 (3-4) (2010) 147–154.
[25] F. Demet Ince, H.Y. Ellidag, M. Koseoglu, N. Simsek, H. Yalcin, M.O. Zengin, The comparison of automated urine analyzers with manual microscopic examination for urinalysis automated urine analyzers and manual urinalysis, Pract. Lab. Med. 5 (2016) 14–20.
[26] Mahmoud Al Ahmad, Zeina Al Natour, Farah Mustafa, Tahir A. Rizvi, Electrical characterization of normal and cancer cells, IEEE Access 6 (2018) 25979–25986.
[27] M. Wolf, R. Gulich, P. Lunkenheimer, A. Loidl, Broadband dielectric spectroscopy on human blood, Biochim. Biophys. Acta 1810 (8) (2011) 727–740.
[28] P.S. Mun, H.N. Ting, T.A. Ong, C.M. Wong, K.H. Ng, Y.B. Chong, Study of dielectric properties of proteinuria between 0.2 GHz and 50 GHz, PLoS One 10 (6) (2015), e0130011.
[29] H.N. Ting, P.S. Mun, T.A. Ong, Y.B. Chong, K.H. Ng, Dielectric properties of urine for diabetes mellitus and chronic kidney disease between 0.2 GHz and 50 GHz, in: Proceedings of the *World Congress on Medical Physics and Biomedical Engineering*, 51, Canada, Toronto, 2015, pp. 1257–1260.
[30] A. Lonappan, V. Hamsakkutty, G. Bindu, J. Jacob, V. Thomas, K. Mathew, Dielectric properties of human urine at microwave frequencies, Microw. Opt. Technol. Lett. 42 (6) (2004) 500–503.
[31] A. Lonappan, G. Bindu, V. Thomas, J. Jacob, C. Rajasekaran, K.T. Mathew, Diagnosis of diabetes mellitus using microwaves, J. Electromagn. Waves Appl. 21 (10) (2007) 1393–1401.
[32] P.S. Mun, H.N. Ting, Y.B. Chong, T.A. Ong, Dielectric properties of glycosuria at 0.2–50 GHZ using microwave spectroscopy, J. Electromagn. Waves Appl. (2015) 1–15.
[33] P. S Mun, H.N. Ting, S.M. Mirhassani, T.A. Ong, C. M Wong, Y.B. Chong, Prediction of chronic kidney disease using urinary dielectric properties and support vector machine, J. Microw. Power Electromagn. Energy 50 (3) (2016) 201–213.
[34] T.A. Larsen, W. Gujer, Separated management of anthropogenic nutrient solutions (human urine), Water Sci. Technol. 34 (3-4) (1996) 87–94.
[35] I. Fittschen, H.H. Hahn, Characterization of the municipal wastewater and a preliminary comparison with liquid cattle excretion, Water Sci. Technol. 38 (6) (1998) 9–16.
[36] H.L.T. Mobley, R.P. Hausinger, Microbial ureases: significance, regulation, and molecular characterization, Microbiol. Rev. 53 (1) (1989) 85–108.
[37] Z. Liu, Q. Zhao, K. Wang, D. Lee, W. Qiu, J. Wang, Urea hydrolysis and recovery of nitrogen and phosphorous as MAP from stale human urine, J. Environ. Sci. 20 (8) (2008) 1018–1024.
[38] P. Froom, B. Bieganiec, Z. Ehrenrich, M. Barak, Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips, Clin. Chem. 46 (9) (2000) 1384–1386.
[39] X. You, M. Misra, S. Gregori, A.K. Mohanty, Preparation of an electric double layer capacitor (EDLC) using Miscanthus-derived biocarbon, ACS Sustain. Chem. Eng. 6 (1) (2018).

[40] S. Abdalla S, S.S. Al-Ameer, S.H. Al-Magaishi, Electrical properties with relaxation through human blood, Biomicrofluidics 4 (3) (2010) 34101.
[41] G.M. Cooper, The Cell: A Molecular Approach, second ed., Sinauer Associates, Sunderland (MA), 2000.
[42] Heloise Pöckel Fernandes, Carlos Lenz Cesar, Maria de Lourdes Barjas-Castro, Electrical properties of the red blood cell membrane and immunohematological investigation, Rev. Bras. Hematol. Hemoter. 33 (4) (2011) 297–301.
[43] Wenfei Bo, Jingchao Tang, Yang Yang, Jialu Ma, Qi Huang, Lianghao Guo, Zhanliang Wang, Zhe Wu, Baoqing Zeng, Yubin Gonga, A numerical study for dielectric constant profile of aqueous solvent in ionic solution radiated by high-intensity electric pulses, AIP Adv. 8 (Issue 11) (2018) 115217.
[44] W.F. van Gunsteren, H.J.C. Berendsen, Algorithms for brownian dynamics, Int. J. Inter. Chem. Phys. 45 (3) (1982) 637–647.
[45] Yizhak Marcus, Effect of ions on the structure of water: structure making and breaking, Chem. Rev. 109 (3) (2009) 1346–1370.