Biosensor diagnosis of urinary tract infections: a path to better treatment?

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Abstract

Urinary tract infection (UTI) is among the most common bacterial infections and poses a significant healthcare burden. The standard culture-based diagnosis of UTI has a typical delay of two to three days. In the absence of definitive microbiological diagnosis at the point of care, physicians frequently initiate empirical broad-spectrum antibiotic treatment, which has contributed to the emergence of resistant pathogens. Biosensors are emerging as a powerful diagnostic platform for infectious diseases. Similar to how blood glucose sensors revolutionized the management of diabetes and pregnancy tests are now conducted at home, biosensors are poised to significantly improve UTI diagnosis. Biosensors are amenable to integration with microfluidic technology for point-of-care applications. This review focuses on promising biosensor technology for UTI diagnosis, including pathogen identification and antimicrobial susceptibility testing and hurdles in the translation of biosensor technology from bench to bedside.

Are better diagnostics needed for urinary tract infections?

Infectious diseases remain one of the greatest challenges in global health. A key barrier towards improving the management of infectious diseases is the absence of rapid and accurate diagnostic information to direct treatment decisions at the point of care (POC). Urinary tract infection (UTI) is among the most common bacterial infections and poses a significant healthcare burden [1]. Almost half of all people will experience a UTI at some point in their lives, and those who suffer from recurrent, complicated UTI may have more than three episodes of infection per year [2–6]. As the most common healthcare-associated infection, UTI accounts for more than 30% of infections reported by acute care hospitals [7–9]. Similar to most bacterial infections, diagnosis of UTI depends on culturing the clinical sample in a centralized clinical laboratory, which has a typical delay of two to three days from sample acquisition to delivery of the culture and susceptibility results. This delay is due to the need for sample transport to centralized laboratories and the time required for bacteria to grow on artificial media for phenotypic identification.

In general, UTI is defined as the presence of urinary symptoms (e.g. frequency, dysuria, and pyuria) and 10^4 cfu/ml or greater uropathogens in urine. Depending on the clinical scenario and the method of urine collection, however, this definition may vary [10]. *E. coli* is the most common cause of UTI, accounting for up to 70% of community-acquired and 50% of hospital-associated UTI's [1]. Other important uropathogens include *Proteus mirabilis, Pseudomonas aeruginosa, Klebisella pneumoniae*, and *Enterococcus* species.

UTI often presents as a clinical conundrum. It is rarely fatal, yet highly morbid and affects all patient demographics. Although effective treatments are available, the associated urinary symptoms are nonspecific and overlap with numerous other non-infectious entities. Furthermore, the presence of bacteria in urine does not always necessitate treatment, yet differentiation of *asymptomatic bacteriuria* from UTI is subjective, particularly in patients with urinary catheters and other neurological or anatomical impairments of the bladder. These challenges, coupled with the inherent delay of urine culture, contribute to widespread mis- and overuse of antibiotics, which has accelerated the selection of resistant pathogens and decreased the lifespan of antibiotics [11]. The rate of UTI caused by *E. coli* resistant to trimethoprim-sulfamethoxazole is typically greater than 30% and resistance to fluoroquinolones ranges from 11% to greater than 50% in some patient populations [12–16]. Infections caused by multi-drug resistant pathogens such as extended-spectrum beta-lactamase (ESBL) *Enterobacteriaceae* [17], methicillin-resistant *Staphylococcus aureus* (MRSA) [18], and fluoroquinolone-resistant *Pseudomonas* are increasingly seen in the urinary tract and are among the most challenging public health issues today [11].

There is significant interest in developing rapid diagnostics for UTI. Widely adopted in the 1980's, the urine dipstick is a simple POC test to assess leukocyte esterase (produced by white blood cells) and nitrite (found in urine in the presence of nitrate reducing bacteria) [19]. More recently, automated platforms of urinary flow cytometry capable of rapid detection of bacteria, white blood cells, red blood cells, epithelial cells, casts, crystals, yeasts and spermatozoa, have been developed and widely adopted by centralized laboratories [20,21]. Although rapid, these technologies do not provide microbiological diagnosis and susceptibility information, which remains as the cornerstone of diagnosis, particularly in settings of complicated UTI [22]. Modern clinical microbiology laboratories utilize high throughput systems (e.g. Vitek) that monitor bacterial CO_2 production for antimicrobial susceptibility testing (AST) [23,24]. These high throughput instruments have semi-automated sample processing, but remains relatively slow and not amenable for POC use.

The ability to obtain rapid, definitive POC diagnosis of UTI will have an enormous favorable impact on its management: timely antibiotic treatment could be initiated and imprecise empirical treatment obviated. The new generation of biosensors based on micro- and nanotechnologies offer the possibility of highly sensitive molecular diagnosis within a compact platform and low power consumption suitable for POC applications. Analogous to how the blood glucose sensors revolutionized the management of diabetes and lateral flow tests have enabled home pregnancy testing, biosensors hold the promise to personalize the treatment of UTI and potentially improve its outcomes. Here, we will highlight recent development in molecular UTI diagnostics based on biosensors. Drawing on the parallel between other successful biosensors in clinical use today, we will discuss the hurdles that remain to translate biosensors for UTI applications. Many comprehensive reviews of the rapidly advancing field of biosensors are available for the interested reader [25–33].

Biosensors basics

A biosensor is any device or system capable of detecting a biological entity, ranging from lateral flow test strips for pregnancy testing [34] to cell-based sensors using B-lymphocytes

to detect pathogens [35]. In the simplest sense, a biosensor is composed of a *recognition element* and a signal *transducer* (Figure 1). Binding of the target (*analyte*) to the recognition element leads to generation of a measurable signal (e.g. electrons, light, mass effect) that is then detected by the transducer. For quantitative detection, the magnitude of the signal is proportional to the analyte concentration. Common examples of recognition elements include antibodies, enzymes, receptors, nucleic acids, aptamers, and other synthetic molecules. Common transducers include electrodes for electrochemical sensors and CCD cameras for optical sensors [25,26,28,30,32]. Recent technological advances have led to development of ultrasensitive transducers such as avalanche photodiodes and nanostructure field-effect transistors, which are capable of detecting down to the single molecule level [26,36]. To improve the detection sensitivity, a secondary recognition element (e.g. detector antibody) is frequently used in a 'sandwich' format and is labeled with signaling molecules (e.g. enzymes, fluorophores, nanoparticles, or quantum dots) to further amplify the signal and obviate the need for direct labeling of the analyte.

What can UTI biosensors learn from the glucose sensor: a model of translation

Almost 50 years after its first description and 30 years after introduction in the current form, the portable blood glucose sensor stands out as the most successful biosensor in commercial use today. Glucose sensors represent over 85% of the $7 billion market on biosensors [27,37]. The glucose sensor is an example of electrochemical sensor, in which the enzyme glucose oxidase serves as the recognition element that is immobilized on an electrode. The oxidation of glucose in the blood by glucose oxidase produces measurable electroactive species detected by the electronic interface [37]. The binding site of the enzyme provides exquisite specificity for glucose and so the measured signal is proportional its blood concentration. By contrast, the home pregnancy test is a lateral flow assay in which binding of a monoclonal antibody against human choriogonadotropin (hCG) in urine generates a visible color change [38]. For multiplex assays, the most well known example that is widely used in emergency departments and intensive care units is the i-STAT, which is a handheld analyzer for common blood chemistry parameters [39]. It is an integrated electrochemical sensor with a set of electrodes of differing electrolyte specificity.

These examples illustrate the crucial aspects of the successful biosensors in the clinical arena—*portability, rapidity, and cost-effectiveness*—in comparison with their macro-scale counterparts. The epidemic scale of diabetes worldwide, the evidence that strict control of blood glucose improves clinical outcome, and the relative low cost have driven the success of the glucose sensors [40,41]. The simplicity of depositing a drop of blood or urine onto the sensor for rapid readout with minimal or no manipulation by the end-user is another key feature. In the setting of a defined clinical need, the simplicity and cost effectiveness of these biosensors allowed them to gain wide acceptance. The features of these assays that make them successful can serve as a model to follow for emerging applications of biosensors for molecular diagnostics, including UTI.

Several issues need to be considered to develop biosensor applications for UTI, including sample preparation and matrix management. Furthermore, given the multitude of potential pathogens and polymicrobial UTI in certain settings, the assay needs to have multiplexing capability. In contrast to blood glucose or urine hCG, detecting the target analytes for UTI require multi-step sample preparation steps, including pipetting (i.e. reagent transfer and mixing), centrifugation (separation and concentration) and washing. Although easily performed in a laboratory setting, they are not practical in POC settings. If the target analyte is intracellular (i.e. nucleic acids), the additional step of bacterial lysis is required. For nucleic acid targets at low concentration, intermediate enrichment steps such as polymerase

chain reaction (PCR) may be needed to amplify the target prior to detection [42]. While PCR can be used for sensitive detection of nucleic acids, it has shortcomings including additional sample preparation steps to remove potential inhibitors and false positives from urine that may contain low levels of skin flora.

The sensitivity of a biosensor can be limited by other factors, such as non-specific binding and the matrix effect. Particularly for clinical applications, the type of biological *matrix* (e.g. urine, blood, saliva) can significantly impact sample preparation steps and sensor performance. Many of the constituents in urine can vary over a broad range [43,44]. For example, urine pH can range from 4.5 to 9, and healthy urine has very few cells, whereas an infected sample can have concentrations of bacteria and white blood cells greater than 10^8 cells/ml.

Specifically for UTI, a successful biosensor needs to meet the following criteria: i) The ability to definitively rule out infection; ii) The assay needs to be fast, within the POC time frame to effect treatment planning; iii) Automation of the sample preparation with minimal intervention from the end-user ('plug and play'); iv) Robust assay protocol compatible with urine matrix effect; v) Incorporation of pathogen identification with antimicrobial susceptibility testing; and vi) Versatile to be adaptable for the different pathogen profiles in different clinical scenarios.

Rapid diagnostics for UTI – pathogen identification

An example of a biosensor for uropathogen identification is the UTI Sensor Array [45–48]. The sensor-platform is based on an electrochemical sensor array customized with bacterial specific DNA probes as recognition elements. Each of the 16 sensors is modified with a surface layer step called self-assembled monolayer to allow versatility in surface modification and reduce background noise [49,50]. A library of the DNA probes targeting the most common uropathogens is immobilized on the sensor surface [45,47]. The detection protocol is based on conversion of hybridization events into quantifiable electrochemical signals.

Figure 2 shows the detection schematic of the UTI Sensor Array. The urine sample is first lysed. Then the bacterial 16S rRNA is detected by sandwich hybridization of capture and detector oligonucleotide probe pairs. 16S rRNA is an ideal target for pathogen identification because it is one of the most abundant molecules in bacteria and has sequences that are highly conserved as well as sequences unique to individual species [51,52]. The relative abundance of the 16S rRNA (approximately 10,000 copies per cell) precludes the need for nucleic acid amplification [51]. The capture probe is immobilized to the sensor surface and the detector probe is free in solution. The fluorescien-labeled detector probed can bind to an anti-fluorescien conjugated with horseradish peroxidase (HRP). The signal is then generated by the redox reaction catalyzed by HRP with H_2O_2 as the substrate and tetramethylbenzidine (TMB) as the electron mediator [53]. This multiplex assay uses universal probe targeting 16S rRNA sequences conserved in all bacterial species, as well as genus- and species-specific probes, allowing for detection of all bacteria via hybridization to the universal probe and refined typing of the most common causative agents. The UTI Sensor Array currently has an overall detection limit of 10^4 cfu/ml, which is within the clinical cutoff compared to urine culture [47]. In testing with patient urine samples, the UTI Sensor Array had 92% overall sensitivity and 97% specificity for pathogen detection compared to urine culture [47,48].

In addition to the UTI Sensor Array, several other technology platforms for rapid molecular diagnostics have been recently described. PCR-based approaches to pathogen identification also show promise for rapid molecular diagnostic for infectious diseases [54]. Recently

Lehmann *et al.* demonstrated direct real-time PCR for identification of pathogens from patient urine samples [55,56]. Using real-time the PCR primers they designed for uropathogen detection this assay had a sensitivity and specificity of 90% and 87%, respectively. For detection of intact bacteria, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) have been described [57,58]. For microorganisms MALDI-TOF MS spectra can be used to differentiate genus and species and potentially even differentiate strains of an organism. Ferreira *et al.* have applied MALDI-TOF MS to the detection of pathogens from urine samples [59–61]. Still at the early stage of technology validation, these technologies share the challenges of implementation at the POC settings.

Antimicrobial susceptibility testing (AST)

Because of the diversity of potential pathogens in the urinary tract, pathogen identification assays need to be complemented by AST in order to select appropriate antibiotic for optimal treatment. For the current standard clinical laboratory analysis pathogens must first be cultured and isolated for AST. The pathogen is then grown in the absence and presence of the antibiotics. Growth in the presence of an antibiotic indicates resistance. The phenotypic assay has been robust because it remains reliably independent of the genetic basis of antibiotic resistance.

An alternative approach is to use a genetic approach based on PCR of known resistant-conferring genes or high throughput microarray of known genetic mechanisms of resistance [62]. PCR-based AST has been clinically successful for identification and differentiation of methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* [63,64]. An important shortcoming of genotypic approach is that genetic mechanisms of AST is constantly evolving and may not necessarily manifest as genetic mutations.

Recently, we described a biosensor-based AST (b-AST) which combines the versatility of the phenotypic assay with genotypic specificity of the 16S rRNA probes [48]. In this assay, following pathogen identification, 16S rRNA is utilized as a bacterial growth marker for phenotypic AST (Figure 3). Each electrode on the UTI Sensor Array biosensor is tethered with an oligonucleotide probe specific to the bacteria of interest and used to measure growth of the pathogen under different antibiotic conditions. Biosensor signals from samples incubated with an antibiotic were comparable in magnitude to biosensors signals from samples incubated without antibiotic, indicating comparable growth and thus antibiotic resistance. In laboratory testing with patient samples, b-AST was overall 94% [48], which is within close proximity of the maximum error rate allowed for a new AST to be considered for FDA approval [65]. The main advantage of the biosensor approach over standard AST is time. Since the biosensor specifically detected the pathogen of interest, pathogen isolation through overnight plating is not necessary. The overall assay time currently is at 3.5 hours, compared to over 18 hours for standard AST (Figure 3) [48]. The b-AST approach is inherently compatible with microfluidics, which facilitates the implementation at the POC and allows on-chip sample preparation.

Sample preparation is a technology bottleneck

A major limitation of many current biosensor assays and molecular diagnostics is the reliance on manual sample preparation steps. Although many existing 'lab-on-a-chip' platforms have small dimensions, they still required various ancillary equipment to be operational, effectively functioning as a 'chip-in-a-lab' [66–68]. To enable automated sample preparation at POC, microfluidics technology, or fluidic manipulation at the micron scale, is the core technology that will integrate the reagent transfer, target isolation, and sample mixing steps in a multi-layered cartridge containing channels, valves, and reagent

reservoirs [67,69–72]. Another related technology to further simplify sample preparation is electrokinetics, which allows direct manipulation of the bulk fluid or selective cells and molecules for pumping, mixing, concentration, and separation in microfluidic systems. An inherent advantage of electrokinetics is its compatibility with both optical and electrochemical detection as well as other microfluidic components [73,74].

Biosensors integrated with microfluidic cartridge have recently been described [42,66]. These disposable cartridges have majority of the necessary components for sample handling with dimensions comparable to a credit card. The cartridge can be inserted into a portable reader device. Electrochemical sensors are well suited for integration with microfluidic systems. Perhaps not surprisingly, both the glucose sensor and the i-STAT are electrochemical sensors, illustrating their compatibility with POC applications.

The future outlook

In the last five years, biosensor diagnosis for UTI has moved beyond proof-on-concept stage into validation phase with clinical samples, with development of assays for rapid molecular pathogen identification and AST. The UTI Sensor Array offers a promising technology platform without the need for nucleic acid amplification. The current technology bottleneck is at sample preparation and system integration, which is crucial for the technology to be used in decentralized settings such as clinicians' office and emergency departments.

The short-term goal of the rapid UTI diagnostics is not to eliminate, but to complement clinical laboratories. Clinical laboratories will still be essential particularly when the rapid test indicates unusual pathogens or unusual susceptibility profiles. Patients occasionally present with UTI caused by presence of a fastidious, anaerobic bacteria that are missed by the methods of routine culture [75,76]. Many of the rapid diagnostics for pathogen detection have the potential to identify the presence of a pathogen in these situations and direct culture conditions such as anaerobic incubation in the laboratory.

The versatility of the biosensors and the potential for multiplexing also raises the possibility that the future UTI diagnostics based on biosensors, in addition to being faster, will be more informative than the current approach. For example, the degree of host response may differ in patients with asymptomatic bacteriuria compared to true UTI, and may be determined by measuring host immunity proteins in urine. There is considerable interest in the identification and measurement of protein biomarkers that indicate the severity of UTI and to differentiate infections from asymptomatic bacteriuria [77]. Addition of protein biomarker analysis to the biosensor assay could even further improve its diagnostic capability [50].

It is interesting to note that the initial introduction of glucose sensors was met with considerable skepticism over whether it would be useful or could ever be cost effective [78,79]. For biosensor diagnosis of UTI—or any POC molecular diagnostic tests for infectious diseases—to succeed, stakeholders including patients, clinicians, and third party payers will need to carefully assess the utility and the cost effectiveness of such technology. Test strips for the glucose sensor and home pregnancy tests cost about $5. The charge for urine culture can range from $95 to over $200 [80]. If mass produced, UTI biosensors may only be modestly more expensive than glucose strips or pregnancy tests due to increased complexity and likely less expensive than standard urine culture. Additional cost savings will likely come from decrease utilization of broad spectrum antibiotics in both inpatient and outpatient settings.

Conclusions

UTI is a common bacterial infection that affects all patient demographics. There is a significant need for improved diagnostics, including pathogen identification and antimicrobial susceptibility profiling. Evidence-based treatment plans can therefore be implemented and judicious usage of antibiotics applied. Biosensors offer a promising approach to deliver highly sensitive molecular diagnostics testing in POC settings. With continuing technology advancements and clinical acceptance, they will potentially lead to a paradigm-shift for UTI diagnosis and treatment and serve as a model for other common infectious diseases.

Acknowledgments

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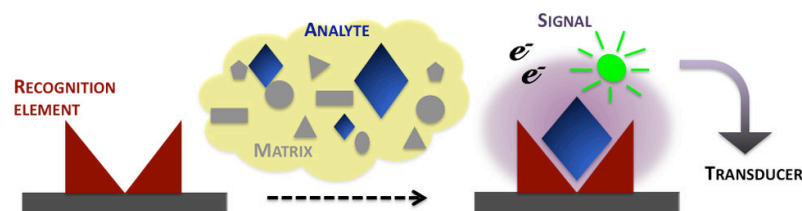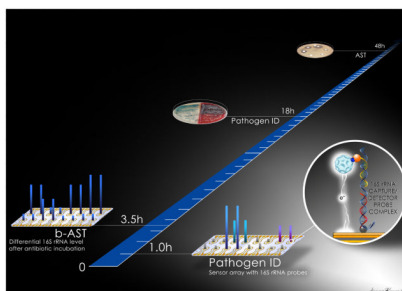
Figure 1.
A biosensor is a molecular sensing device composed of a *recognition element* and *transducer*. Specific binding of the target *analyte* to the recognition element generates a measurable *signal* (e.g. light, electrical current) that is detectable via the transducer (e.g. CCD camera, photodiode, electrode). Common examples of recognition elements include enzymes, antibodies, and DNA that are able bind specifically to target analytes, including glucose, ions, protein, and nucleic acids which are indicative of the state of health or disease. The *matrix* is the biological medium (e.g. blood, urine, saliva) with varying biochemical parameters and nonspecific cells and molecules that may impact the performance of the biosensor.
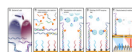
Figure 2.
Multiplex pathogen detection scheme using an array of 16 electrochemical biosensors (UTI Sensor Array). Each sensor is composed of 3 electrodes: working, reference, and counter. A. Lysis of pathogens in urine samples releases the 16S rRNA target. B. Hybridization of the 16S rRNA targets with a cocktail of detector probes labeled with fluorescein (orange sphere). C. Deposition of the 16S rRNA-detector probe hybrid onto the sensor surface (working electrode) for sandwich hybridization with the capture probes. Biotin-labeled (gray sphere) capture probes of different specificities are tethered to the surface of each sensor. D. Binding of the anti-fluorescein horseradish peroxidase (HRP) enzyme tag to the sandwich hybrid. E. Oxidation of the HRP substrate H_2O_2 and electron mediator TMB under a fixed voltage generates an electroreduction current. The magnitude of the signal output corresponds to the starting concentration for each pathogen. The limit of detection is 10^4 cfu/ml. Modified from Reference [47], with permission from copyright holder, Elsevier.

Figure 3.
Biosensor diagnosis of UTI is significantly faster than standard culture-based approach.
Pathogen identification (ID) and biosensor-based antimicrobial susceptibility test (b-AST)
may be completed within 1 and 3.5 hours, respectively, compared to 1–3 days for standard
culture. Reprint with permission from copyright holder, Elsevier [48].