Contents lists available at ScienceDirect

Analytica Chimica Acta

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

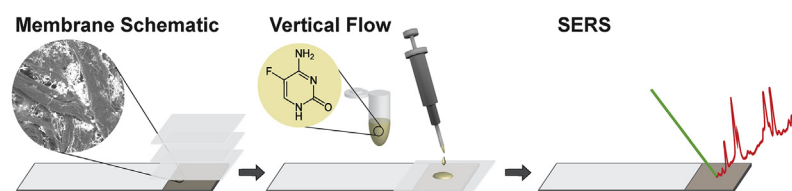# Vertical-flow paper SERS system for therapeutic drug monitoring of flucytosine in serum

Adam G. Berger, Stephen M. Restaino, Ian M. White*

Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA

GRAPHICAL ABSTRACT

ABSTRACT

A number of life-saving drugs require therapeutic drug monitoring (TDM) for safe and effective use. Currently, however, TDM is performed using sophisticated analytical techniques relegated to central labs, increasing the cost per test and time to answer. Here, using a novel vertical flow membrane system with inkjet-printed surface enhanced Raman sensors, along with a portable spectrometer, we demonstrate a low cost and easy to use device to quantify levels of flucytosine, an antifungal that requires TDM for effective patient care, from undiluted human serum. To our knowledge, this work represents the first report of a passive vertical flow sample cleanup method with surface enhanced Raman detection. We first investigated and optimized the parameters of the vertical flow system for the detection of flucytosine in spiked serum samples. Then, using an optimized vertical-flow system utilizing nitrocellulose membranes and a paper SERS sensor, we achieved detection of down to 10 μg mL^{-1} flucytosine in undiluted serum, with quantitative detection across the entire therapeutic range. This system reduces the assay time to about 15 min, far quicker than the current gold standards. We anticipate that this novel system will enable near-patient therapeutic drug monitoring, leading to the safe and effective administration of a number of life-saving drugs. Furthermore, it will spawn the development of SERS detection systems capable of separating target analytes from real-world biological matrices.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Recommendations to monitor the real-time physiological load of therapeutic drugs in patients, referred to as therapeutic drug monitoring (TDM), have been increasing with the move towards precision medicine [1–3]. In particular, TDM may be necessary for therapeutics with a narrow window of efficacy or for therapeutics with a high variability in renal elimination among patients. Classes of drugs that typically warrant TDM include antimicrobials [4–8], chemotherapeutics [9,10], and anti-psychotics [11,12], among others.

One of the most documented cases in which TDM can dramatically improve patient outcomes is that of the antifungal drug flucytosine (5-fluorocytosine - 5FC). Flucytosine is primarily used to treat infections with *Candida* species and *Cryptococcus neoformans* [13,14], and is critical for treatment of Cryptococcal Meningitis [4,15]. In fungal cells, cytosine deaminase converts the 5FC form into the functional 5-fluorouracil (5FU) form, which causes RNA miscoding and inhibits DNA synthesis, thus interfering with protein synthesis [5,15,16]. While it is effective in the appropriate blood concentration, toxic side effects can occur in high doses, including bone marrow suppression, hepatotoxicity, and gastrointestinal toxicity [16–18]. One study suggests that up to 40% of patients receiving 5FC develop hepatotoxicity [16], and in a study of nearly 200 patients, 91% of patients showed bone marrow suppression after four weeks of treatment [18]. The commonly accepted threshold for toxicity is a serum concentration of 100 $\mu g \; mL^{-1}$ [4,5,15–19]. Meanwhile, the minimum inhibitory concentration (MIC) for fungal infections is on the order of 10 $\mu g \; mL^{-1}$ [15], and commonly recommended doses for a range of fungal infections are often between 20 $\mu g \; mL^{-1}$ and 80 $\mu g \; mL^{-1}$, depending on the targeted species [13,14,16,17,20]. 5FC dosage is further complicated by the fact that there is high variability in renal elimination of 5FC [13]. To illustrate this, a study of over 200 patients showed that only about 20% of patients had 5FC levels within the expected range, while about 40% had levels below the target dose and about 40% had levels above the target dose, of which about 10% were toxic [14]. Moreover, for Cryptococcal Meningoencephalitis, 5FC is often administered as a cocktail with amphotericin B (AmB) [19], which can cause renal impairment, thus adding to the variability of 5FC load in patients. As a result, the need for TDM of flucytosine is widely recognized [4,13,15–17].

Current methods for TDM do not meet the need for a quick, inexpensive, portable, multiplex-capable, near real-time, and sensitive determination of serum drug concentration. Available methods include microbiological assays, high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) [4,13,21]. Microbiological assays require manual steps, culturing, and are vulnerable to interference when combination antifungal therapy is used [4,13]. Standard analytical chemistry techniques (e.g., LC/MS) are more sensitive and allow rapid quantification even when combination antifungal therapy is used; however, they are expensive and require complex equipment operated by skilled technicians. Thus, samples must be transported to a central laboratory facility for analysis and queued [4,13]. It is clear that a near-patient solution for flucytosine TDM is necessary to improve patient outcomes.

Surface enhanced Raman spectroscopy (SERS) has been investigated as an alternative to traditional analytical chemistry techniques for chemical and biomolecular analysis. SERS is based upon Raman scattering, the inelastic scattering of light unique to molecular vibrations inherent in the molecule. Though Raman scattering is a relatively rare event, noble metal nanostructures provide plasmonic enhancement to the intensity of Raman scattering [22–24]. While in most cases SERS cannot compete with HPLC/MS for sensitivity and specificity in a central lab setting, SERS may be capable of meeting applications away from the central lab, including analysis in the field and in near-patient settings, aided by the emergence of high-performing portable and handheld Raman spectrometers. To date, SERS has been examined for TDM of the chemotherapeutics methotrexate and 5-fluorouracil [25–27], aminoglycosides [28], the anti-epileptic valproic acid [29], and a host of IV drugs [30]; however, reports of SERS for TDM from applicable bodily fluids, such as serum or saliva are still lacking, as are pragmatic demonstrations applicable to detection in the field.

While miniaturized spectrometers can lead to portability, SERS can only be applied for TDM in near-patient settings if sample processing is simplified, as serum, plasma, or whole blood samples present challenges for direct SERS analysis. Components within serum foul the plasmonic surface and thus inhibit direct interactions with the targeted therapeutic. New sample preparation methods integrated with SERS sensors must be developed. In particular, proposed techniques must not require labor- or equipment-intensive intervention steps to meet the needs of portability and ease-of-use.

Traditional SERS sensors were not designed with these constraints in mind. Historically, SERS sensors have been fabricated onto rigid substrates using relatively sophisticated synthesis techniques, leading to high chip costs or limited mass production capabilities. Alongside the cost of production, rigid substrates provide only basic analytic functionality and therefore require off-chip equipment and manual steps for sample acquisition and processing. To address these limitations, our group developed inkjet-printed paper-based SERS sensors as low cost and easy to use sensors for chemical and biomolecular analysis [31–34]. While paper-based SERS sensors were first reported 30 years ago [35,36], it has only emerged as a realistic solution for chemical analytics within the last 5 years, starting with reports of high-performing substrates fabricated with inkjet printing [34], screen printing [37], and soaking [38].

The cost of printed sensors on paper substrates is clearly an advantage for near-patient applications, but equally important are the sample acquisition and cleanup capabilities. We demonstrated the inherent capabilities of paper SERS sensors to collect samples from surfaces via swabbing or from liquid via dipping [32]. We also reported integrated sample cleanup through detection of melamine in infant formula by using chromatography on a PVDF membrane that had been decorated with silver nanoparticles via inkjet fabrication [33].

In this work, we demonstrate a new scheme to monitor flucytosine in undiluted serum, which can be broadly applied in many other TDM applications. Two previous reports have demonstrated SERS for TDM applications in diluted serum. One report measured a chemotherapeutic in serum [26], though the limit of detection prevented quantification across the therapeutic range, likely because of variability introduced from the lack of sample cleanup. Another report demonstrated detection of tobramycin, an aminoglycoside antibiotic, in serum with a 1000-fold dilution, but the detection at this low dilution was only possible with the aid of a Raman reporter [28]. Furthermore, the need for a dilution likely inhibits near-patient applications because of the need for precise intervention steps. Our solution for TDM with undiluted serum samples is illustrated in Fig. 1. A sample droplet is applied to a membrane selected to trap serum components while transmitting the therapeutic. Beneath the filtering membrane is an inkjet-fabricated paper SERS sensor, which wicks the sample into the sensor from the membrane. The potential fouling agents are trapped in the nitrocellulose, while the 5FC passes through the membrane and adsorbs onto the nanoparticles. The sensor is easily interrogated with a portable Raman spectrometer.

Using this vertical flow design, we report the measurement of 5FC in undiluted serum across the entire therapeutic window. Although vertical flow assays have been shown in the past, to the best of our knowledge none have been used for SERS or for pump-free separation of undiluted serum [39–42]. We first show that utilizing nitrocellulose as the filtering membrane results in the highest discrimination, as other materials may not bind protein as well or may prevent the passage of 5FC. We then optimize the number of nitrocellulose membranes in the vertical flow stack, the sample volume, and the amount of time allowed for vertical flow. In addition, we investigate the impact of diluting the serum sample