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Redox cycling-based amplifying electrochemical sensor for *in situ* clozapine antipsychotic treatment monitoring

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ABSTRACT

Schizophrenia is a lifelong mental disorder with few recent advances in treatment. Clozapine is the most effective antipsychotic for schizophrenia treatment. However, it remains underutilized since frequent blood draws are required to monitor adverse side effects, and maintain clozapine concentrations in a therapeutic range. Micro-system technology utilized towards real-time monitoring of efficacy and safety will enable personalized medicine and better use of this medication. Although work has been reported on clozapine detection using its electrochemical oxidation, no *in situ* monitoring of clozapine has been described. In this work, we present a new concept for clozapine *in situ* sensing based on amplifying its oxidation current. Specifically, we use a biofabricated catechol-modified chitosan redox cycling system to provide a significant amplification of the generated oxidizing current of clozapine through a continuous cycle of clozapine reduction followed by re-oxidation. The amplified signal has improved the signal-to-noise ratio and provided the required limit-of-detection and dynamic range for clinical applications with minimal pre-treatment procedures. The sensor reports on the functionality and sensitivity of clozapine detection between 0.1 and 10 μg/mL. The signal generated by clozapine using the catechol-modified chitosan amplifier has shown to be 3 times greater than the unmodified system. The sensor has the ability to differentiate between clozapine and its metabolite norclozapine, as well as the feasibility to detect clozapine in human serum *in situ* within the required dynamic range for clinically related applications. This new biosensing approach can be further developed towards its integration in miniaturized devices for improved personalized mental health care.

1. Introduction

One of the major challenges facing mental health clinicians is when patients discontinue care due to lack of optimized treatment. This problem has usually been demonstrated in mental health illnesses where chronic conditions require prolonged treatment with harsh and burdensome side effects. For instance, schizophrenia is one of the most complex psychiatric disorders. It is a lifelong illness that affects 1.1% of the population worldwide. The estimated direct and indirect costs of the illness exceeded $60 billion in 2002 [1]. Currently there is no cure for the disorder and lifelong treatment with antipsychotic drugs is recommended [2]. Approximately 30-50% of patients do not respond to primary, first-line antipsychotic treatment [2–4]. Clozapine (CLZ), a second-line antipsychotic, is the most efficacious medication currently available, providing effective treatment for patients who are unresponsive to other antipsychotics. It is also the only antipsychotic drug approved by the Food and Drug Administration (FDA) for treatment-resistant schizophrenia [2,5–10]. Despite its high efficacy, CLZ remains underutilized because of the required frequent and invasive blood draws to monitor adverse side effects such as agranulocytosis (decrease in the

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amount of white blood cells) [11,12]. Frequent CLZ testing for dose titration and efficacy monitoring has been shown to improve treatment outcomes and reduce the risk of toxicity [13–18]. However, this is often not implemented due to the frequent visits and supplementary blood draws required of patients, in addition to the weekly white blood cell counts over the first six months of treatment [19–25]. Miniaturized devices have the potential to revolutionize the way these illnesses are currently being treated, and to improve overall mental health care outcomes. For example, real-time monitoring of CLZ at the point-of-care will provide a rapid in-office means for physicians to monitor CLZ levels and adjust dosages accordingly to reach safe and effective blood levels. This approach will potentially reduce the cost and burden of monitoring, and increase the acceptance of CLZ treatment by patients and prescribers, leading to improved symptom control in patients [9,26,27].

Electrochemical biosensors are based on bioelectrochemical reactions of electro-active species being consumed or generated. By measuring the electrical signal from these reactions, oxidation/reduction of an analyte is detected [28,29]. Portable integrated electrochemical microsystems provide numerous advantages in clinical diagnostics, environmental monitoring and biomedical research fields. These translational technologies can be easily realized using microfabrication technology for the development of on-chip electrochemical microsystems where the sensing electrodes are integrated directly onto the microchip. This approach yields high magnitude signals and relatively low noise, with detection limits that are satisfactory for many practical applications [29,30]. Over the years, the electrochemical activity of CLZ [31,32] and its detection [33–57] were investigated. Still, only a small subset of studies showed electrochemical analysis of CLZ in blood samples [31,42,43,47,49]. Notably, these studies relied on extensive sample pre-treatment procedures prior to the electrochemical measurement; *e.g.* electrode rinsing and testing in a serum-free measurement cells, or deoxygenation with nitrogen. These pre-treatment procedures are mainly due to the increased background signal from electrochemical reactivity and non-specific adsorption of molecules in the serum, decreasing the signal-to-noise ratio and deteriorating the overall performance of the sensor. However, as these procedures add cost, time, and complexity to the system, the utilization for *in situ* real-time CLZ sensing at the point-of-care is impeded.

In this work, we present a new concept for *in situ* CLZ sensing based on an electrochemically-active biomaterial for CLZ oxidation amplification. We leverage the naturally derived polysaccharide chitosan, a versatile biomaterial [58,59], as a scaffold for subsequent modification with electrochemically-active catechol, resulting in a redox cycling system [60–64]. This allows for significant amplification of the generated oxidizing current of CLZ through a continuous cycle of CLZ reduction by the catechol moieties followed by re-oxidation at the electrode. The continuous redox reaction increases the total charge transferred by CLZ oxidation, amplifying the generated electrochemical current and improving the signal-to-noise ratio. This provides the improved sensitivity and detection range required for *in situ* CLZ analysis of clinical samples, *i.e.* blood levels between 0.350 μg/mL and 1 μg/mL for high efficacy and low toxicity [17]. In this work we study the functionality and detection limit of CLZ sensing with the amplification system. We also characterize selectivity with respect to the CLZ principal metabolite norclozapine (NorCLZ). Finally, we demonstrate the feasibility of the sensor to detect CLZ in human serum *in situ* within the therapeutic range. Therefore, the presented biosensing mechanism represents a promising candidate for integration in miniaturized analytical devices with minimal pre-treatment procedures for real-time *in situ* detection of CLZ.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma-Aldrich. Chitosan solution (1%; pH 5–6) was prepared by dissolving chitosan flakes in dilute HCl as previously described [65]. All other chemical solutions were prepared in phosphate buffer (PB; 0.1 M; pH 7).

Characterization of the biosensing mechanism in buffer solution was performed with microfabricated planar square gold electrodes. These electrodes (7.5×7.5 mm^2 electrode area; 20×8 mm^2 chip dimensions; 1 electrode per chip) were fabricated using standard microfabrication techniques. Briefly, 20 nm of chrome and 180 nm of gold were sputtered on 4 inch diameter silicon wafers insulated by 500 nm of PECVD silicon oxide, patterned using photolithography, and diced into chips. Prior to experiments, they were subjected to consecutive cleaning steps using acetone, methanol, isopropanol, piranha solution (25% H_2O_2/75% H_2SO_4), and deionized (DI) water. Human serum testing was performed with gold disk working electrodes (2 mm diameter; CH Instruments, Austin, TX; Polished before use).

2.2. Catechol-modified chitosan system biofabrication

Catechol-modified chitosan films were deposited following recently published protocols [63]. The gold working electrodes were coated with chitosan by immersion in solution and application of 6 A/m^2 cathodic current for 45 seconds using a three-electrode system with platinum foil as a counter electrode and a Ag/AgCl reference electrode with 1 M KCl electrolyte (CH instruments, Austin, TX). Subsequently, the chitosan films were functionalized by immersion in 5 mM catechol in PB and application of 0.6 V (*vs.* Ag/AgCl) anodic potential for 180 seconds, followed by immersion for 5 minutes in DI water to discard unbound catechol. Characterization of amplification dependence on the modification steps was performed by applying both electrochemical modification techniques (chitosan electrodeposition and catechol grafting) for electrodes immersed in either the modification solution or buffer alone, *i.e.* chitosan and catechol solutions were used for the catechol-modified chitosan electrode, chitosan and buffer (no catechol) solutions were used for the chitosan-modified electrode, buffer (no chitosan) and catechol solutions were used for the catechol electrode, and only buffer solutions were used for the unmodified electrode.

2.3. Electrochemical testing

All electrochemical tests were carried out using the three-electrode system described above and with a CHI660D single channel potentiostat from CH Instruments (Austin, TX), except of serum testing where Bio-Logic VSP-300 potentiostat was used (Claix, France; following reproducibility tests of CLZ in PB with the commercial gold disk electrodes). All voltages are denoted *vs.* Ag/AgCl. Catechol-modified chitosan films were initialized through cyclic voltammetry (range −0.4 V to +0.7 V, scan rate of 0.1 V/s, scan resolution of 0.001 V) in 25 μM hexaammineruthenium(III) ($Ru(NH_3)_6$; HARu) and 25 μM 1,1′-ferrocenedimethanol ($Fc(MeOH)_2$) prior to CLZ experiments. This cycle in the presence of oxidizing and reducing mediators decreased the observed background current. This may be attributed to the consumption of remaining unbound catechol molecules. Moreover, the initialization step prior to analyzing CLZ samples provides a means to validate the presence, amplification capability, and reproducibility of the biofabricated catechol-modified chitosan film. Reproducibility tests of the initialization solution with $Fc(MeOH)_2$ for different fabricated catechol-chitosan electrodes (N = 8) resulted averaged