TOWARDS PERSONALIZED MENTAL HEALTHCARE: AN ELECTROCHEMICALLY-AMPLIFIED BIOSENSOR FOR CLOZAPINE ANTIPSYCHOTIC TREATMENT MONITORING

H. Ben-Yoav[1*], T.E. Winkler[1], S.E. Chocron[1], G.R. Costa[1], S.M. Restaino[1], N. Woolsey[1], E. Kim[1], D.L. Kelly[2], G.P. Payne[1], and R. Ghodssi[1*]

[1]University of Maryland, College Park, Maryland, USA and

[2]University of Maryland School of Medicine, Baltimore, Maryland, USA

ABSTRACT

We present the first demonstration of a micro-scale electrochemically-amplifying biosensor for real-time monitoring of the antipsychotic clozapine. The amplification probe consists of a catechol-modified chitosan redox-cycling system, which increases the electrochemical signal generated by clozapine. Modified microelectrodes show a 2.5-fold higher clozapine oxidative current density compared to bare microelectrodes. Chitosan electrodeposition at the milli- and micro-scale yields deposition rates of 5.0±0.8 nm/s and 3.0±0.6 nm/s, respectively. Catechol grafting duration demonstrates positive correlation with the signal amplification, modeled using the oxidizing mediator ferrocenedimethanol. Moreover, the feasibility of the biosensor to distinguish 5, 25, and 50 µM of clozapine is successfully shown.

KEYWORDS: Redox-cycling, Antipsychotic clozapine, Electrochemical amplifier, Biosensor, Mental health

INTRODUCTION

Schizophrenia is a lifelong mental disorder with little recent progress in treatment. Clozapine (CLZ) is the most effective antipsychotic drug for schizophrenia treatment. Yet, it remains underutilized since frequent blood draws are required to maintain drug concentrations in a therapeutic range and monitor adverse side effects. Real-time monitoring of treatment efficacy and safety will enable personalized medicine and better utilization of this medication [1]. Lab-on-a-chip (LOC) devices are a promising solution and provide numerous advantages in clinical diagnostics [2], such as small sample volume, ease of use, fast response, low cost, and high throughput. Although work has been done on CLZ detection, LOC technology has not been utilized to monitor CLZ treatment. Here, we have designed a new chitosan-based electrochemical microchip for real-time analysis of CLZ.

Chitosan is a versatile biomaterial that can be easily integrated into a LOC [3]. By grafting electro-active catechol (Q), a redox cycling system can be formed [4]. Our approach uses the redox system as a biosensor through amplification of the CLZ oxidation signal. Following the diffusion and oxidation of CLZ (E_0=+0.4 V vs. Ag/AgCl) on the electrode surface under overpotential conditions, CLZ is reduced back by the grafted QH_2 moieties, and electrochemically re-oxidized at the electrode (Figures 1a & 1b). A continuous cycle of CLZ reduction in the presence of catechol, followed by CLZ re-oxidation at the electrode, results from the use of CLZ as an oxidizing mediator (Figure 1c). The continuous redox reaction increases the total charge transferred by the oxidation of each CLZ molecule, amplifying the total electrochemical current generated and improving the signal-to-noise ratio. To recover the redox cycling system to the reduced state, a negative potential is applied in the presence of a reducing mediator, Ru^{2+} (Ru^{2+}/Ru^{3+}, E_0=−0.2 V vs. Ag/AgCl). Our previous work [5] involved developing a biosensing mechanism for CLZ detection at the milli-scale. As down-scaling challenges have to be overcome (e.g., low signal-to-noise ratio), this work incorporates the translation, integration, and characterization of the CLZ biosensing system in a micro-scale device designed for CLZ monitoring at the point-of-care.

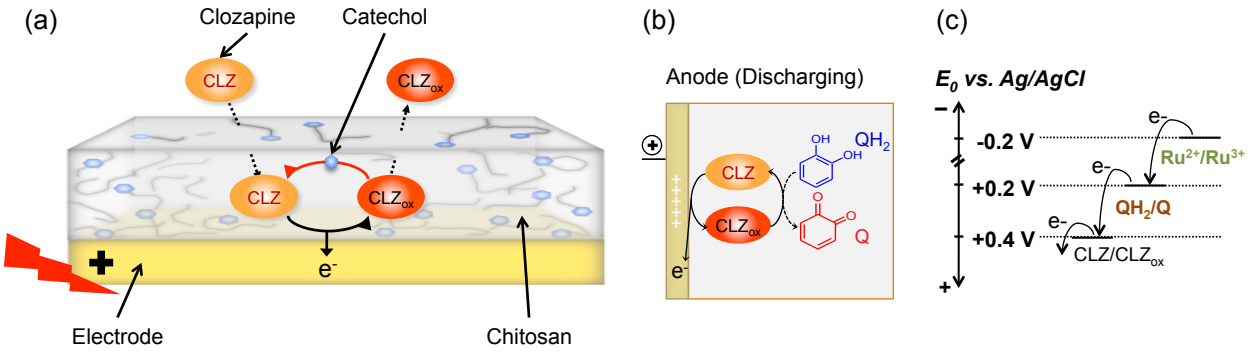

Figure 1: CLZ as an oxidizing mediator in the catechol-chitosan system. (a) Schematic of the CLZ oxidation/reduction cycle for signal amplification. (b) Continuous oxidation of CLZ in the presence of catechol (Q) reduction. (c) CLZ acts as an oxidizing mediator of QH_2, and Ru^{2+} as a reducing mediator regenerating the Q.

EXPERIMENTAL

The micro-scale biosensor comprises 2 parts (Figure 2a): (1) an arrayed electrochemical microchip, and (2) 3 parallel chambers defined with polydimethylsiloxane (PDMS). The microchip is based on 3×3 patterned gold working electrodes (radius of 100 µm for amplification characterization, and 500 µm for biofabrication characterization work) modified from [6]. Each of the three rows further includes a set of on-chip gold counter and pseudo-reference electrodes. The chips are fabricated using established photolithography and wet etching processes, and cleaned with piranha solution prior to use. The PDMS chambers were cut from cured slabs of the material and bonded to the microchips. When milli-scale volume solution was used (for volumes greater than 200 µL), the microchip was dipped in a petri dish for testing.

The chitosan film is biofabricated by application of 1.19 A/m^2 cathodic current density. Subsequently, the chitosan films are grafted with catechol by immersion in 5 mM catechol and application of +0.6 V, followed by immersion for 5 minutes in deionized water (DI) to discard unbound catechol (Figures 2b: no chitosan and catechol electrical modification steps & 2c: catechol-modified chitosan). Analytical solutions contain either 110 µL or 12 mL (for micro- and milli-scales respectively) of 25 µM hexaammineruthenium(III) ($Ru(NH_3)_6$) and either clozapine or ferrocenedimethanol as an oxidizing mediator. All samples are prepared in 0.1 M phosphate buffer (pH 7).

Biofabrication is characterized by varying chitosan deposition times and catechol grafting times. CLZ sensing is recorded with cyclic voltammetry (range of −0.4 V to +0.7 V, scan rate of 0.02 V/s, scan resolution of 0.001 V). Background signals are similarly recorded using buffered solution with only 25 µM $Ru(NH_3)_6$. All electrochemical tests are carried out using a CHI660D single channel potentiostat from CH Instruments (Austin, TX). All voltages are denoted versus the relevant reference electrode half-cell potential. Chitosan film height was determined in a Veeco contact profilometer after drying the hydrogel under nitrogen flow.

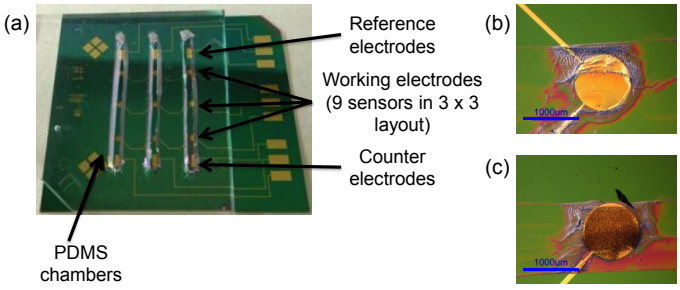

Figure 2: (a) Photograph of the fabricated arrayed electrochemical microchip (3.5 cm × 4 cm), a single microelectrode with (b) no chitosan and catechol modifications (chitosan residues are observed due to chitosan solution in the chambers without electrical current application) and (c) catechol-chitosan modifications (210 seconds chitosan electrodeposition with 150 seconds of catechol grafting).

RESULTS AND DISCUSSION

Initially, the ability of the miniaturized redox cycling system to amplify the electrochemical current generated by CLZ was evaluated. In Figure 3, we present the electrochemical signal of CLZ in the presence and absence of the catechol-modified chitosan system. The amplification is observed at an anodic peak current density of +0.4 V, as expected for CLZ, with a signal 2.5-fold higher compared to an unmodified electrode. This is only marginally lower than the previously determined 3-fold for millimeter-scale electrodes [5].

To obtain better understanding and control of biofabrication parameters, we first studied the chitosan electrodeposition rate for micro- and milli-scale solution volumes. Through profilometry of films with deposition times up to 300 seconds, we determined a linear correlation of film thickness with duration of 3.0±0.6 nm/s for 110 µL and of 5.0±0.8 nm/s for 12 mL- for milli- and micro-scales respectively (Figure 4a). We attribute the difference between milli- and micro- scale to the additional constraints placed on diffusion by the presence of the PDMS chambers, and different distances between the micro- and milli- scale counter and working electrodes.

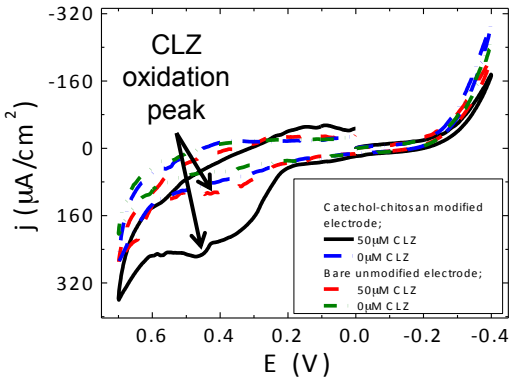

Figure 3: CLZ amplification comparison between modified and unmodified electrodes with a 110 µL solution.

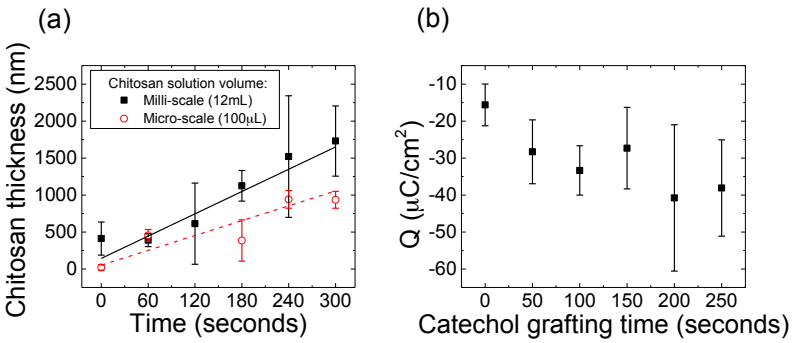

Figure 4: (a) The influence of the electrodeposition duration on the chitosan thickness with milli- and micro-scale solution volumes. (b) The effect of catechol grafting duration on the amplification of ferrocenedimethanol oxidative charge transfer (210 seconds chitosan deposition, 110 µL solution).

We further investigated the impact of the catechol grafting time on amplification performance. These experiments utilized optimized chitosan deposition times of 210 seconds, and solutions with the model oxidizing mediator ferrocenedimethanol. In Figure 4b, we demonstrate a positive relationship be-

tween catechol grafting time (0–250 seconds) and the total oxidative charge generated between 0 and +0.7 V of a cyclic voltammogram. This is expected since more catechol is available to donate electrons to the mediator. The increase in standard deviation with grafting time, however, indicates a trade-off between amplification and accuracy. We speculate this is due to variations in chitosan matrix distribution at the electrode being compounded at longer grafting times.

We show the detection feasibility of 5, 25, and 50 μM CLZ in buffered solutions in Figure 5 (using the biofabrication parameters 210 seconds of chitosan electrodeposition and 50 seconds of catechol grafting). The cyclic voltammograms reveal a negative dose-dependence of the calculated total oxidative charge generated between 0 and +0.7 V of a cyclic voltammogram. These results show the first step towards the development of a microfluidic LOC for CLZ detection. However, they also demonstrate the challenges that arise within micro-scale systems, such as the large deviations observed in CLZ detection. Accurate control of system biofabrication is critical to overcome these challenges; therefore, following the integration of a microfluidic device, a comprehensive characterization of the device architecture (e.g., electrodes and microfluidics geometry), the biofabrication process (e.g., chitosan electrodeposition and catechol grafting steps) and the competence and the biosensing performance (e.g., compatibility with human serum) will be conducted to maximize the amplification performance and accuracy of the biosensor.

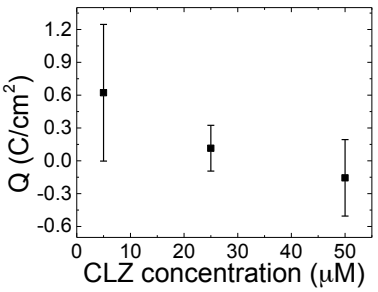

Figure 5: CLZ detection in 110 μL buffer solutions with 5, 25, and 50 μM concentrations (210 seconds chitosan deposition).

CONCLUSION

The biosensor presented here combines the redox cycling system for CLZ signal amplification with the LOC paradigm of real-time, high throughput, and cost-effective detection. The chitosan electrodeposition and the catechol grafting microfabrication steps were studied, and the functionality of the biosensor to detect CLZ was demonstrated.

In future work, we will focus on characterizing the detection limit and the dynamic range of the biosensor, with relation to the required clinical range. Following performance characterization of the device with serum fluids, a flow system will be integrated to realize a fully autonomous micro-system for clinical monitoring of CLZ blood levels in schizophrenia patients. This will allow treatment teams to perform analysis at the point-of-care in a low cost, fast, and straightforward way aiming to guide CLZ dosages within the effective range [7], to decrease the patient's burden, and to personalize medical care.

ACKNOWLEDGEMENTS

The authors would like to thank the Robert W. Deutsch Foundation and the National Science Foundation Maryland Engineering Research Internship Teams (MERIT) and the NSF Grant No. DGE0750616 for financial support. The authors likewise appreciate the support of the Maryland NanoCenter and its FabLab. The authors wish to also thank Dr. Raymond C. Love and the Bioengineering rotation students in MSAL: Bao-Ngoc Nguyen, and Bharath Ramaswamy for the useful discussions.

REFERENCES

[1] D. L. Kelly, J. Kreyenbuhl, R. W. Buchanan, and A. K. Malhotra, "Why Not Clozapine?," *Clin. Schizophr. Relat. Psychoses*, vol. 1, pp. 92-95, Apr. 2007.

[2] H. Craighead, "Future Lab-on-a-Chip Technologies for Interrogating Individual Molecules," *Nature*, vol. 442, pp. 387-393, Jul. 2006.

[3] S. T. Koev, P. H. Dykstra, X. Luo, G. W. Rubloff, W. E. Bentley, G. F. Payne, R. Ghodssi, "Chitosan: An Integrative Biomaterial for Lab-on-a-Chip Devices," *Lab Chip*, vol. 10, pp. 3026-3042, Sep. 2010.

[4] K. M. Gray, E. Kim, L-Q. Wu, Y. Liu, W. E. Bentley, and G. F. Payne, "Biomimetic Fabrication of Information-rich Phenolic-chitosan Films," *Soft Matter*, vol. 7, pp. 9601-9615, Jun. 2011.

[5] H. Ben-Yoav, T. E. Winkler, E. Kim, D. L. Kelly, G. F. Payne, and R. Ghodssi, "Redox Cycling-based Amplifying Electrochemical Biosensor for *In Situ* Clozapine Antipsychotic Treatment Monitoring," Submitted to *Biosens. Bioelectron.*, Aug. 2013.

[6] H. Ben-Yoav, P. H. Dykstra, W. E. Bentley, and R. Ghodssi, "A Microfluidic-based Electrochemical Biochip for Label-free Diffusion-restricted DNA Hybridization Analysis," *Biosens. Bioelectron.*, vol. 38, pp. 114-120, Oct.-Dec. 2012.

[7] A. Stark and J. Scott, "Review of the Use of Clozapine Levels to Guide Treatment and Determine Cause of Death," *Aust. N. Z. J. Psychiat.*, vol. 46, pp. 816-825, Sep. 2012.

CONTACT

* R. Ghodssi, tel: +1-301-4058158; ghodssi@umd.edu
* H. Ben-Yoav, tel: +1-301-4052168; benyoav@umd.edu