TEAM RESULT DOCUMENT

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SensUs

TRUSENSE

Content

1 Summary

TruSense is from Zhejiang University, China, which mainly consists of devoted undergraduates to develop a point-of-care device for the welfare of epilepsy patients. Despite the pandemic of the coronavirus, our endeavor yielded TruSensor, an electrochemical biosensor that integrates molecular biology, electrochemistry, engineering and computer science technologies. Though conceptual, core technologies of the biosensor have been validated with experiments. By dripping a drop of blood onto the chip and gently inserting it into the portable device we developed, VPA blood concentration can be measured easily and promptly, which provides useful guidance for administration. Along with the device, we have also designed a user-friendly Android app, enabling real-time update of testing results from the device as well as offering visualized statistical results. To put the biosensor into practical use, we have comprehensively analyzed China's medical market and developed a business model which shows that there is a promising future for our biosensor.

2 Biosensor System and Assay

2.1 Molecular recognition and assay reagents

We develop an electrochemical biosensor for free valproate (VPA) detection in the plasma sample. Free VPA in the plasma sample is recognized by VPA-specific molecular imprinted polymer (MIP) on a carbon screen printed electrode (SPE), which allows only VPA to enter the cavity and be adsorbed to the electrode surface. The SPE has been previously modified with cytochrome P450 (CYP450), an enzyme capable of catalyzing VPA to form 4-OH-VPA. As one of the natural enzymes for catalyzing VPA degradation, CYP450 has been known to retain its biological activity and catalytic ability when modified onto the electrode surface.

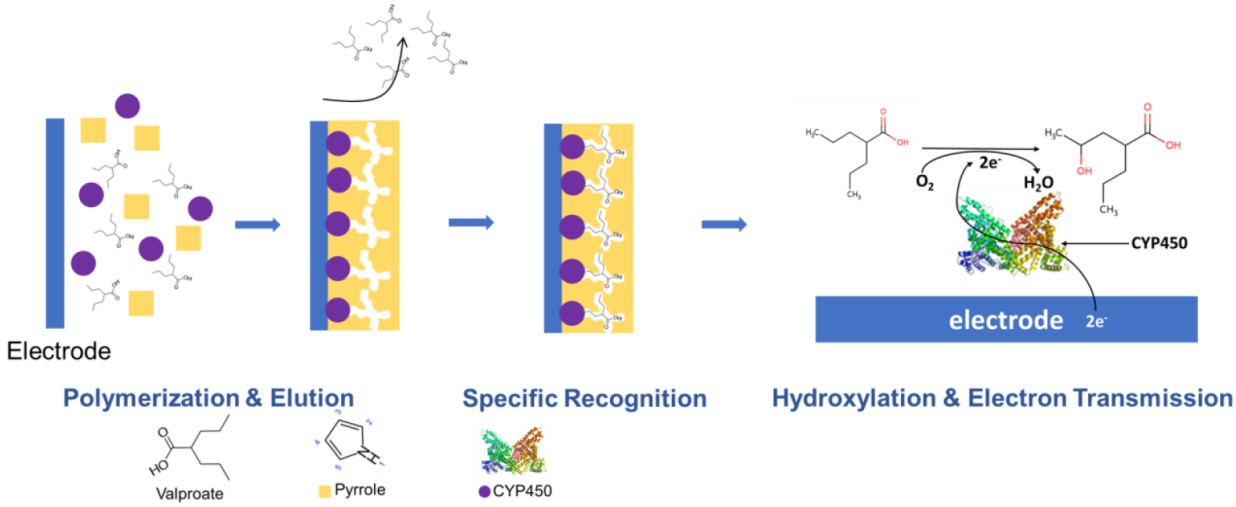


Fig. 1. Fabrication process of a VPA-sensitive electrode and molecular recognition process of VPA on electrode surface.

As VPA enters MIP attached to the CYP450-modified electrode surface, subsequent hydroxylation of VPA by CYP450 results in direct electron transfer from CYP450 to SPE, which can be detected by the organic electrochemical transistor (OECT) devices, generating electric signals. In addition, to enhance detection sensitivity, a multi-walled carbon nanotube (MWCNT), a multi-layered graphene cylinder structure featured with efficient electron transmission and high biomolecule affinity, is modified to cover the surface of the carbon electrode.

2.2 Physical transduction

Organic electrochemical transistor (OECT) is a semiconductor device that generates current signals depending on the input electric field. Its features of simple fabrication, high sensitivity and low voltage operation make it an ideal device for the real-time detection of VPA. Gate, source, drain, semiconductor layer, insulation layer and substrate are the major components of OECT. For depletion mode OECTs, the appliance of gate voltage would force cations in the electrolyte to enter or exit the semiconductor layer and change its conductivity, resulting in the alteration of the drain current, which is eventually converted to the concentration of VPA.
PEDOT: PSS is a p-type semiconductor and its conductivity is proportional to the density of holes within. When VPA is oxidized by CYP450 on the gate in plasma, the gate voltage of OECT increases, forcing cations to enter PEDOT: PSS and compensating the holes, undermining its conductivity, and eventually leading to the drop of

the current. Therefore, a high concentration of VPA in plasma induces a low OECT drain current, while a low concentration evokes a relatively high current, making it possible to detect VPA sensitively and rapidly.

2.3 Cartridge technology

We use poly ethylene terephthalate (PET) plate as the base plate of our disposable device. In our case, all three electrodes were made of carbon paste and printed on PET substrate using a screen-printing technique. The channel between the drain and source had a width of 0.25 mm and a length of 6 mm and was spin-coated with PEDOT: PSS. PEDOT: PSS doped with 5% DMSO was used as a semiconductor and spin-coated onto the channel. The OECTs were then dried in vacuum and heated in nitrogen to fix all components. An insulating layer was painted across the drain, source and gate on top of the channel, using the same technology as printing electrodes. The drain-source channel and three electrodes are to be covered by a plasma layer, with surface channels on it.

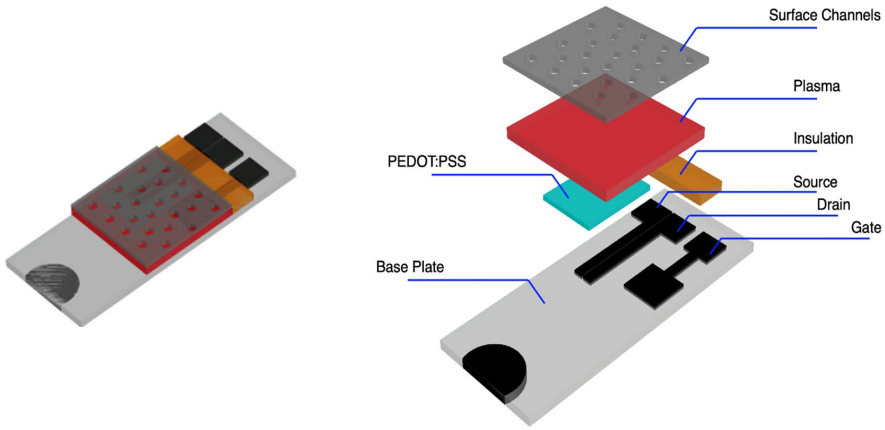


Fig. 2. The structure of the disposable device (OECT)

2.4 Reader instrument and user interaction

To provide patients with most convenience, we designed an integrated, automated and user-friendly reader instrument. The device is 12cm×8cm×2cm in dimensions, featured with a 2-inches LED screen and two buttons for power control and measurement management, respectively. The simple arrangement of screen and buttons allows patients to handle measurement safely and easily, offering direct and rapid data acquisition. During testing, patients are expected to follow a 4-step simple procedure: pierce on the finger with provided, pre-sterilized needle; squeeze out a drop of blood onto the cartridge, insert the cartridge into the sensor and push the buttons. Powered by a built-in battery, OECT detects the chemical signal and converts it into the current signal, which is collected with LMP91000 and processed by an in-house written program in STC12-C5604AD. The final output is both displayed on the screen as concentrations and sent to TruM app on a mobile device through a Bluetooth connection.

The app TruM enables the real-time update of testing results from the device. In the case of temporal connection disability, the outputs can also be added into the app manually with ease. Alongside with the testing results, descriptions of daily symptoms are supported to be recorded, facilitating comprehensive tracking of patients' health status. In addition, our app offers visualized statistic results of recent VPA blood concentrations. To preserve the documents in the long term, users can create a personal account to upload all the records to our cloud server and synchronize them to multiple mobile devices.

3 Technological Feasibility

3.1 Molecular imprinted polymer (MIP) based on polypyrrole

MIP refers to a type of polymer that is synthesized in the presence of template molecules. After removing the molecules, cavities would be left in the polymer matrix with an affinity for the template. Polypyrrole (PPy) is considered to be an important conjugated polymer which exhibits controlled electric conductivity and good stability over a wide pH range. We choose it as the polymer matrix for molecular recognition of VPA molecule. We performed basic experiments investigating how polypyrrole membrane respond to VPA molecule, and the polypyrrole MIP conjugated on gold electrode is responsive to VPA at low concentration. It is verified by decrease in peak current of CV curve during CV scans in 10 mM $[Fe(CN)_6]^{3-/4-}$ solution before and after immersing a MIP electrode in VPA solution (Fig. 3), indicating that VPA could enter the polymer and block the cavities. Thus, the polymer is potentially capable of specific free VPA recognition in plasma sample.

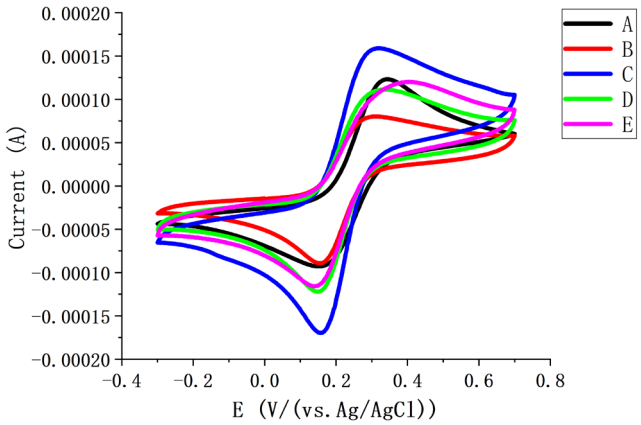


Fig. 3. CV measurements (scan rate of 100 mV/s) (A) bare Au; (B) PPy MIP/Au; (C) PPy MIP/Au, gently washed in 0.01M PBS; (D) PPy MIP/Au, incubated in 0.01M VPA; (E) PPy MIP/Au, washed again; (F) PPy MIP/Au incubated in 0.005M VPA.

3.2 CYP450 integration on the electrode

We tested the amperometric performance of 3 electrodes with/without the presence of VPA in 0.1M PBS solution using the cyclic voltammetry method, they are: SPE, SPE drop-casted with MWCNT (MWCNT-SPE), SPE drop-casted with MWCNT and CYP450 (CYP-MWCNT-SPE). The results support that CYP450 has been successfully integrated onto the electrode with retained redox activity. And the corresponding electrode is sensitive to VPA solution (in 0.01M PBS) with different VPA concentrations, and with a detection limit at about 0.1 mM. The CYP450-functionalized electrode can response to different VPA concentrations ranging from 0.13 to 0.67 mM. Additionally, the peak currents appear to be strongly linear-related with the VPA concentrations.

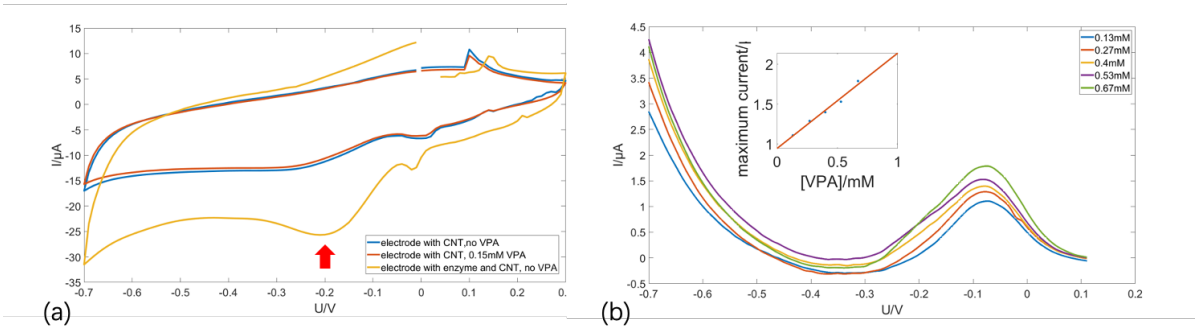


Fig. 4. (a) CV graph of MWCNT-SPE in the presence (blue)/absence(red) of VPA in PBS solution (0.1M), and CYP-MWCNT-SPE (yellow) in the absence of VPA in PBS (red arrow indicates VPA oxidation peak catalyzed by CYP450); **(b)** CV graph of CYP-MWCNT-SPE in VPA solution with gradient concentration, which shows linearity between peak current and VPA concentration.

3.3 OECT device has high stability and detecting ability

Like organic field-effect transistor (OFET), OECTs act like a switch, in which the gate voltage (input signal) controls the drain current (output signal). Chemical reactions that involve direct electron transfer with the electrode would change the gate voltage. Therefore, the drain current is indictive of gate voltage, which reflects the analyte concentration in solution. As for now, the OECT circuit we make have a good performance on testing the concentration of H_2O_2, which would be catalyzed into H_2O by Pt on gate.

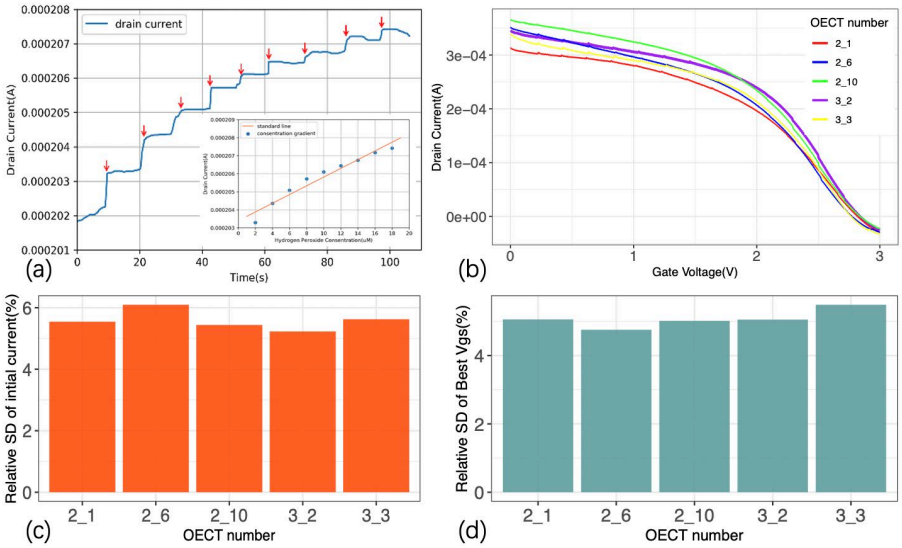


Fig. 1. experimental proof for the sensitivity and repeatability of OECT. **(a)** drain current staircase, by gradual addition of H_2O_2 at 10s intervals, which shows linearity with H_2O_2 concentration (r^2 = 0.9474); **(b)** feature curve of 5 OECT device; **(c, d)** relative SD of best VGS, another feature parameter of OECT.

Upon testing H_2O_2 concentrations, the OECT circuit (gate electrode modified with Pt nanoparticle) shows a high sensitivity (Fig. 5 (a)), with a detection limit at around 2uM. We further improved the crafts of OECT construction, and succeed in yielding good repeatability when we identify the initial current and the most-sensitive gate voltage, which are important parameters for OECT device (Fig. 5 (b, c, d)). In conclusion, the OECT device we fabricated has been proved effective, sensitive and stable, and is able to be integrated into the final biosensor for VPA detection.

3.4 Overall feasibility

The biosensor meets the need of real-time measurement of VPA concentration in the plasma sample, with a high accuracy (Fig. 4 (a, b)) as well as a low detection limit of 0.12mM (Fig. 4 (b)). Despite the requirement of 3 minutes incubation time needed for VPA diffusion through the MIP to the electrode surface, the following electrochemical assay is rather time-efficient using amperometric method wherein only 10s are needed (Fig. 5 (a)). Thus, the measurement could be completed within 4 minutes altogether. The estimated time required for the measurement and the corresponding accuracy is as well supported by a series of previous researches (2, 5, 10). Additionally, our molecular simulation revealed that bound VPA is buried in albumin, thus it cannot enter the cavities, which means only free VPA could be recognized. (Fig. 8) To summarize, we conclude that our biosensor would be a qualified and promising POCT device.

4 Originality

4.1 Written by the team

MIP that specifically recognizes VPA based on poly-pyrrole has already been investigated by Simin Sabah et al. in 2006. Inspired by their study, we adopt pyrrole as functional monomer for the synthesis of MIP, but with a different electropolymerization approach mentioned above. Parameters involved in the polymerization has been optimized that favors CYP450 bioactivity in our own case.

As an amplifier for electrochemical signal, OECT has been widely adopted in the design of electrochemical biosensor which requires high accuracy and fast response. We have manufactured the device independently, including designing the structure of OECT, fabricating the electrodes and printing the insulation. We have also optimized several parameters involved in the manufacture and have produced highly repeatable OECT device. To our best knowledge, it is the first time to integrate CYP450 into an electrode to detect VPA concentrations. Although the bioelectronic and bioactivity properties of CYP450 on electrode has been investigated before and the enzyme is commonly fixed onto an electrode via physical adhesion to carbon nanotube drop-cast, we have adopted a novel approach and successfully produced a CYP450-modified carbon paste electrode with a high valproate detection sensitivity.

Signature 1: Yuyang Yuan

Signature 2: Tianyu Li

4.2 Written by the supervisor

This current TruSense team is the best I have supervised. They visited hospitals and interviewed front-line doctors to fully understand the clinical situations of epilepsy in China. They did thorough literature research, and came up with many exciting ideas. After in-depth considerations and many internal debates, finally they decided to pursue a very innovative strategy: to develop a molecular imprinted polymer (MIP)- cytochrome P450 (CYP450) enzyme- organic electrochemical transistor (OECT)-based VPA biosensor. MIP has been used to detect VPA before. But it is the first time to successfully combine MIP with CYP450, thus significantly increasing the detection sensitivity and specificity of free plasma VPA, while OECT is the best signal processing and amplification element for this specially designed sensing component. I truly believe that this innovative TruSensor is indeed the embodiment of this talented team's hardworking, bravery and dedication.

Signature 3: Liquan Huang

5 Translation Potential

5.1 Business model canvas

Key partners	Key activities	Value Proposition	Custom relationship	Customer Segments
• Equipment manufactures • Companies specialized in electrochemical detection and apparatus development • Investors • Strategic partners • Experts in epilepsy	• R&D on a robust, fast and adaptable bioassay for blood drug concentration monitoring • Miniaturization of the testing apparatus • User-friendly APP development • Close cooperation with key partners • Multiple marketing strategies **Key resources** • Key laboratories • Experienced research mentors • Accurate and efficient testing experience • Innovation and entrepreneurship guidance	• Cheap POCT drug-monitoring device with great accuracy • Adaptive test chips that allow wide applications • Simple operation and minimized interactions • High time/financial efficiency • Better communication between doctors and patients • Online diagnosis	• Optimization of diagnosis and treatment scheme • Technical support • Provide instructions for instrument operation • Client services **Channels** • Doctor's recommendation • Online marketing • Off-line promotional activities • Multi-center research platform	• Epileptic patients • Family doctors • Primary-care medical institutions • R&D centers • Medical devices companies
Cost structure • R&D • Production cost of sensors and strips • G&A • Sales & Marketing			**Revenue stream** • Fund-raising income • Government financial support • Sales revenue sensors and strips	

5.2 Stakeholder desirability

In China, the incidence of epilepsy is about 7‰, among which more than 9 million patients need reasonable treatment. And this number continues growing in the speed of 400,000 per year. As a chronic disease, epilepsy patients have to take anti-epilepsy drugs (AEDs) to suppress seizures. Since each individual respond differently to the same drug, doctors can't determine the appropriate dosage for the patients based on experience merely and it's necessary to monitor the dynamics of such drugs in blood to avoid severe side effect and maximize the treatment efficiency. 4 major stakeholders are involved in the diagnosis and medication procedure of an epilepsy patient (Appendix Fig. 10): the hospital, pharmaceuticals corporation, patient and family, and insurance company. According to the main pain points of different stakeholders listed in (Appendix Table 2), the protruding drawbacks of the existing medical modal are the tedious test process and heavy financial burden for patients.

Our solution is TruSensor, a point-of-care device that can monitor the blood concentration of VPA in epilepsy patients. With only 15μL of blood on the testing chip and easy operation, users can read their blood concentration and record their health status on our app, which is connected to the device via Bluetooth. Based on users' profiles of the blood concentration-efficacy, doctors could optimize the treatments efficiently, which drastically cut down the relevant costs of the patients and the workload of doctors. The TruSensor, designed initially for the valproate (VPA), a first-line AED, can be adaptable to other small molecules that need monitoring in the plasma due to its special mechanism.

5.2.1 Market requirement

China's medical devices market scale has reached 92.35 billion USD in 2019. In the past five years, its expanding rate has been above the global growth rate (Fig. 6 (a)). However, the ratio of market scale of medical device to that of drug is 1:4.5 in China, while it's 1:1 in the US, suggesting a considerable incremental space of medical device industry in China.

China's AED market also has also been developing rapidly in recent years. From 2008 to 2016, the size of the domestic AED market has increased by six-fold from 79.26 million USD to 525 million USD. The overall market

was buoyed mainly by sales of VPA. From 2015 to 2017, the sales of VPA topped the list of antiepileptic drugs, occupying 34% of the market. The sales of VPA in hospital in 2017 exceeded 221 million USD, an increase of over 45%. (Fig. 6 (b))

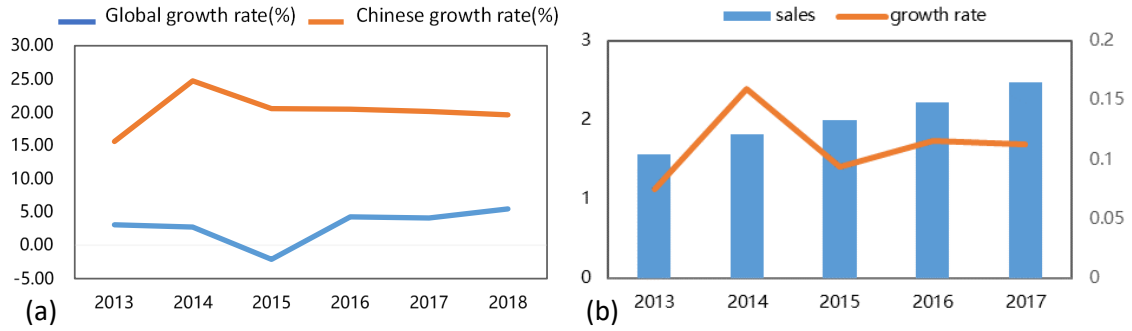


Fig. 6. (a) Global and Chinese medical device market growth rate; **(b)** The amount and growth rate of VPA sales in public hospitals in 16 key cities in China

5.2.2 China's special market position

In China, primary-care medical institutions account for the largest proportion (95%) of medical system in China. (Appendix Fig. 11 (a)) Contrast to the first-tier hospitals which enjoys rich medical resources and use diverse AEDs for different subtypes, VPA occupies a dominant position in primary-care medical institutions due to its broad-spectrum and wide application to nearly all subtypes of epilepsy. Furthermore, China is implementing the policy of graded diagnosis and treatment as well as the family doctor system, which fully supports the development of primary-care medical institutions in China and promises to release a POCT market space of more than 1.76 billion USD as forecasted (Appendix Fig. 11 (b)). With the enormous exiting market scale and substantial potential to be tapped, China's POCT market is fertile to breed the development of TruSensor.

5.2.3 Value proposition

TruSense are devoted to develop user-friendly point-of-care devices to promote personalized medicine, relieve the heavy load of medical system and ease the strained patient-doctor relationship.

5.3 Business Feasibility

To make our business feasible, there're three critical factors: powerful supports, appropriate marketing strategy and wise promotion strategy.

5.3.1 Support

Our business plan has received supports from epilepsy experts (Appendix Table 3), investors, and most importantly the Headquarters Economy Park for Alumni Corporation of Zhejiang University, which integrates the Zhejiang University's disciplines, talents and alumni resources and benefits from the government policy support and successfully incubate many research projects from laboratory to the market. TruSense gets a financial support of 3000 USD from the Headquarters Economy Park this year and has the access to the network of Alumni Enterprises where we can find our potential partners, e.g. downstream industrial cooperators, electrochemical-specialized Enterprises, etc.

5.3.2 Marketing strategy

We are planning to sell our sensors in two forms, as integrated VPA biosensor and as modules. The integrated,

automatic VPA biosensor allows fast and accurate detection of VPA with minimized user interactions. We would directly sell biosensors by establishing a sales network centered in Zhejiang Province, eastern China and rely on our multi center research platform to further boost the influence of our products. What's more, the screen-printed electrode and electric circuit we produced can be applied to various testing situations. With the help of our industrial, we would be able to produce them in large amount and sell them to interested companies or individuals.

5.3.3 Promotion strategy

The core of our promotion strategy is "developing" and "transforming". With the support from art-of-state research groups in Zhejiang University, we would devote into developing fresh and advance technology, which enables faster and more accurate detection of small molecules. The technology is then put into applications and in turn, promote the business development, further expand the influence of our products. Such organic combination of technology development and technological transformation make it possible for our cooperation to grow constantly. We aim to build a comprehensive, multi center research platform eventually.

5.4 Financial viability

In terms of the financial support, we estimate the first round of financing to be 220,000 USD, which will be spent on sales, promotion, product development and management. We set the price of sensors at 69 USD each and test chips 2 USD each accordance with the estimated production cost of the sensors (40 USD per unit) and chips (1 USD per piece). Based on the number of epilepsy patients and the market share of VPA in China, our target population can reach 2.04 million. We predict that the market coverage in the following 5 years could reach 0.5%, 0.6%, 0.9%, 1.2%, and 1.5%, respectively. We are confident to have a net profit in the fifth year, which is expected to be 1.39 million USD. (Fig. 7, Table 1)

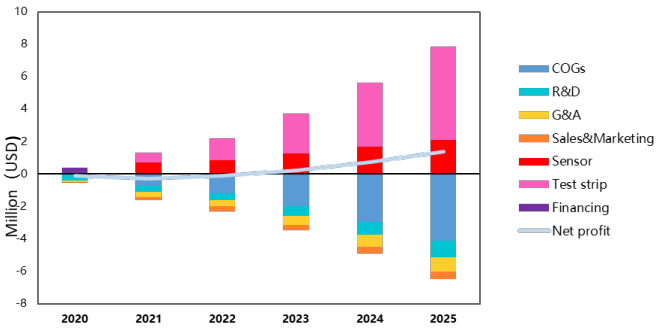


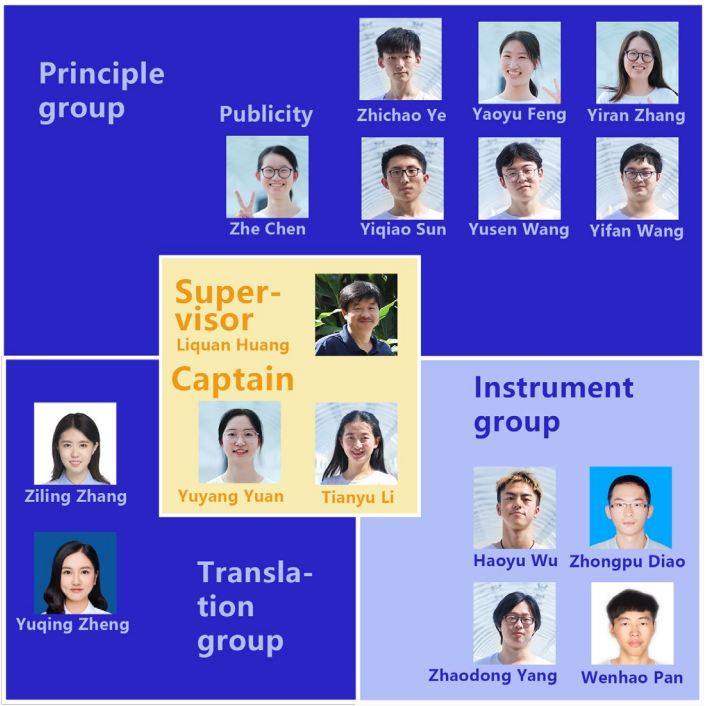
Fig. 7. 5-year cash flow.

	Year	2020	2021	2022	2023	2024	2025
Revenues	Sensor		703800	844560	1266840	1689120	2111200
	Test strip		612000	1346400	2448000	3916800	5752800
	Financing	400000	0	0	0	0	0
Costs	COGs	0	714000	1162800	1958400	2937600	4100400
	G&A	106600	288900	380200	554500	713500	906300
	R&D	388000	397000	449000	609000	815000	1015000
	Sales&Marketing	15400	216000	279600	340300	406200	451600
Net profit		-110000	-300100	-80640	252640	733620	1390900

Table 1. 5-year net profit prediction

6 Team and Support

6.1 Contributions of the Team Members



Supervisor & Coach & Captains:

Prof. Liquan Huang is the team supervisor. He is very responsible and have offered us valuable guidance.

Tianyu Li is the team coach, who offered major experimental support, as well as giving insightful suggestions to build a better biosensing system and to overcome technological difficulties.

Yuyang Yuan is captain of TruSense2020. She managed the teamwork, led the discussion and was responsible for communicating with the SensUs Organization. She has also contributed to the synthetizing of MIP.

Translation group:

Yuqing Zheng is in charge of the Translation group and she also managed the team finance.

Ziling Zhang is excelled in making business plan and giving high quality pitches.

(**Ian Chew** and **Yishan Hu** also made contributions in the early stage of the program.)

Principle group:

Yuyao Feng spent a large amount of time in the lab even during the early stage of pandemic. She suggested using pyrrole as functional monomers to fabricate MIP, which remarkably promoted the process.

Zhichao Ye is meticulous about the lab work and proved the feasibility of MIP.

Yiran Zhang majors in Chemistry and used her professional knowledge to facilitate the lab work.

YiQiao Sun and Yusen Wang concentrated on the electrochemical properties of VPA and creatively used CYP450 to catalyze the oxidation of VPA. They also tried to simulate the behavior of CYP450 and the interaction between VPA and plasma proteins.

Zhe Chen is good at results analysis and has participated in experiments of various aspects. More importantly, she is an outstanding artist and a wise social media manager.

Yifan Wang worked out a plan B using antibodies, and mainly participated in the experiments of CYP450.

Instrument group:

Haoyu Wu bridged the principle group and the instrument group and conducted focused on the test of OECT device.

Zhaodong Yang designed the OECT that accustomed to the upstream bioassay and optimized the fabrication process.

Zhongpu Diao developed the android application and the Bluetooth connection module of the biosensor.

Wenhao Pan mainly worked on the signal processing module.

6.2 Sponsors

SensUs 浙江大学 ZHEJIANG UNIVERSITY

Provide experimental funds and technical support

Provide support to practice and research

7 Final Remarks

Being involved in “zero to one” product development is challenging, but also inspiring. In the past year, our team spare no effort to develop a rapid, sensitive biosensor specific for VPA. Unfortunately, due to the influence of COVID-19, some work is left unfinished, including the performance tests in the plasma samples. We’d like to continue with technological experiments in the following months and eventually launch our completed biosensor, which would be ready for commercialization.

TruSense2020 has received various help during our research progress. We want to thank Professor Liquan Huang and Professor Bo Liang for their generous and insightful instructions. We are also grateful to Zhejiang University and our sponsors for equipment support and financial support. At last, we’d like to thank SensUs for providing such a valuable opportunity for us to discover, and to communicate.

8 References

(1) Baj-Rossi, C.; Rezzonico Jost, T.; Cavallini, A.; Grassi, F.; De Micheli, G.; Carrara, S. Continuous Monitoring of Naproxen by a Cytochrome P450-Based Electrochemical Sensor. *Biosens. Bioelectron.* **2014**, *53*, 283–287. https://doi.org/10.1016/j.bios.2013.09.058.

(2) Parlak, O.; Keene, S. T.; Marais, A.; Curto, V. F.; Salleo, A. Molecularly Selective Nanoporous Membrane-Based Wearable Organic Electrochemical Device for Noninvasive Cortisol Sensing. *Sci. Adv.* **2018**, *4* (7), eaar2904. https://doi.org/10.1126/sciadv.aar2904.

(3) Liao, C.; Zhang, M.; Niu, L.; Zheng, Z.; Yan, F. Organic Electrochemical Transistors with Graphene-Modified Gate Electrodes for Highly Sensitive and Selective Dopamine Sensors. *J Mater Chem B* **2014**, *2* (2), 191–200. https://doi.org/10.1039/C3TB21079K.

(4) Bostick, C. D.; Mukhopadhyay, S.; Pecht, I.; Sheves, M.; Cahen, D.; Lederman, D. Protein Bioelectronics: A Review of What We Do and Do Not Know. *Rep. Prog. Phys.* **2018**, *81* (2), 026601. https://doi.org/10.1088/1361-6633/aa85f2.

(5) Gualandi, I.; Tonelli, D.; Mariani, F.; Scavetta, E.; Marzocchi, M.; Fraboni, B. Selective Detection of Dopamine with an All PEDOT:PSS Organic Electrochemical Transistor. *Sci. Rep.* **2016**, *6* (1), 35419. https://doi.org/10.1038/srep35419.

(6) Bernards, D. A.; Malliaras, G. G. Steady-State and Transient Behavior of Organic Electrochemical Transistors. *Adv. Funct. Mater.* **2007**, *17* (17), 3538–3544. https://doi.org/10.1002/adfm.200601239.

(8) Schneider, E.; Clark, D. S. Cytochrome P450 (CYP) Enzymes and the Development of CYP Biosensors. *Biosens. Bioelectron.* **2013**, *39* (1), 1–13. https://doi.org/10.1016/j.bios.2012.05.043.

(9) Rivnay, J.; Inal, S.; Salleo, A.; Owens, R. M.; Berggren, M.; Malliaras, G. G. Organic Electrochemical Transistors. *Nat. Rev. Mater.* **2018**, *3* (2), 17086. https://doi.org/10.1038/natrevmats.2017.86.

(10) Carrara, S.; Cavallini, A.; Garg, A.; De Micheli, G. Dynamical Spot Queries to Improve Specificity in P450s Based Multi-Drugs Monitoring. In *2009 ICME International Conference on Complex Medical Engineering*; IEEE: Tempe, AZ, 2009; pp 1–6. https://doi.org/10.1109/ICCME.2009.4906648.

9 Appendix

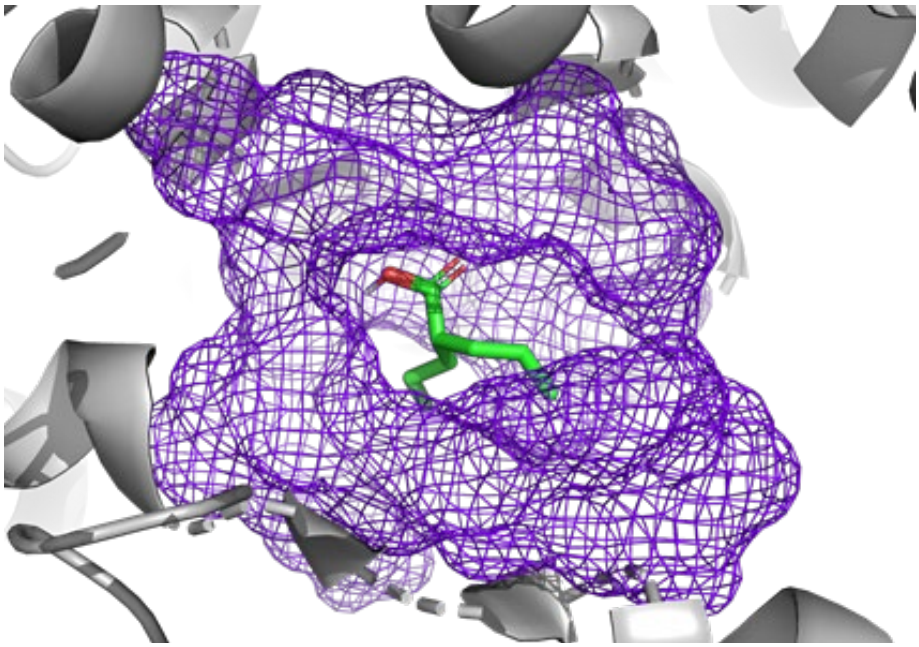
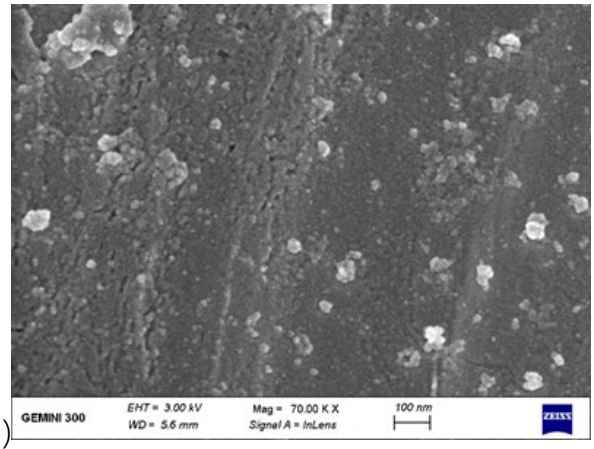


Fig. 8. Binding site of VPA and albumin (simulated by Autodock).

(a)



(b)

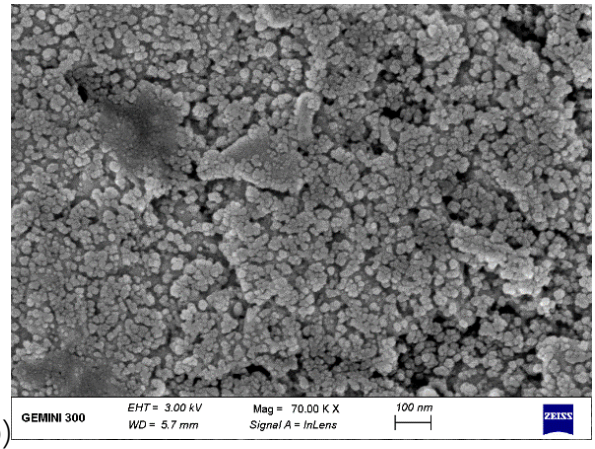


Fig. 9. Scanning Electron Microscope (SEM). **(a)** bare Au electrode; **(b)** MIP@Au.

Fig. 10. The timeline from pre-diagnosis to post-diagnosis of a typical epileptic in China.

Table 2. The table below shows that the pain points of 4 major stakeholders separately, followed by our opportunities.

	Hospital	Pharmaceutical company	Patients	Insurance company
Pain points	☹ Overloaded ☹ Cumbersome testing devices	☹ Insufficient competitiveness of domestic drugs	☹ Tedious test process ☹ Serious side effects caused by inaccurate detection ☹ Financial burden	☹ Ethical risk
Opportunities	☺ Reduce doctors' workload ☺ Optimize the testing process	☺ Cooperative Sales Strategy	☺ Economical POCT devices ☺ Accurate and fast sensing assay ☺ User-friendly app to boost patient-doctor communication	☺ Provide a subsidiary tool for applicant situation statement

Table 3. Interview with an epilepsy expert.

Title of activity	An Interview with a neurologist specialized in epilepsy
Local Community	The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China
Date	2020.03.27
Preparation time	One hour to sort out the interview outline and one day to get in contact with the doctor.
Type of activity	Interview
Abstract	Interview a neurologist specialized in epilepsy and get useful information about the clinical therapy on epilepsy and the use of VPA and so on.
Objective of activity	Deep investigation into the VPA market in China.
Promotion	E-mail and the social software WeChat.
Partners	A neurologist specialized in epilepsy.
Contact person	Yuyang Yuan, Tianyu Li
Evaluation method	Sort out the results of the interview and use the results as part of the guidance of the business model.

Evaluation	
Number of participants	3
Lessons learnt	● VPA is the first choice for comprehensive epilepsy, while lamotrigine is the first choice for partial epilepsy. ● Among all epilepsy patients, the proportion of comprehensive epilepsy is no more than 15%. More than 95% of adult epilepsy is partial epilepsy, and comprehensive epilepsy is more common in children. Therefore, children use more VPA. (in The First Affiliated Hospital, Zhejiang University School of Medicine) ● Neurologists attach little importance to the concentration of VPA in blood, because patients would acquire tolerance to VPA during the long-term therapy, and a high concentration of VPA can have a better effect. ● It is necessary for pregnant women to test the blood concentration. The drug metabolism rate of pregnant women is faster, and the blood concentration may decrease more rapidly, reducing the effect of epilepsy treatment. However, VPA can't be used on pregnant women because it can cause teratogenesis. ● It will take at least one day (in advanced hospitals like FAHZU and longer in community hospitals) for the patient to get the results of the blood drug concentration test in the hospital, because the hospital has to wait for the samples to accumulate and send them for examination in batches. ● Biosensor for fast detection of VPA blood concentration can be used and promoted ① in hospitals which use the VPA as the only drug for epilepsy. (For example, some hospitals can't afford multiple kinds of antiepileptic and only use VPA because it's broad-spectrum.) ② when the patient uses other drugs metabolized by liver while using VPA, it is necessary to detect the blood concentration of VPA, which is an inhibitor of liver drug enzyme. But clinically, doctors often choose to avoid VPA and use other antiepileptic drugs. ● Any clinical technology improvement is valuable and can definitely make a difference and the key is to find out the proper target.
Recommendations	The idea of SensUs is great! University students indeed can use their creativity and passion to make a difference and create a better life for the patients.

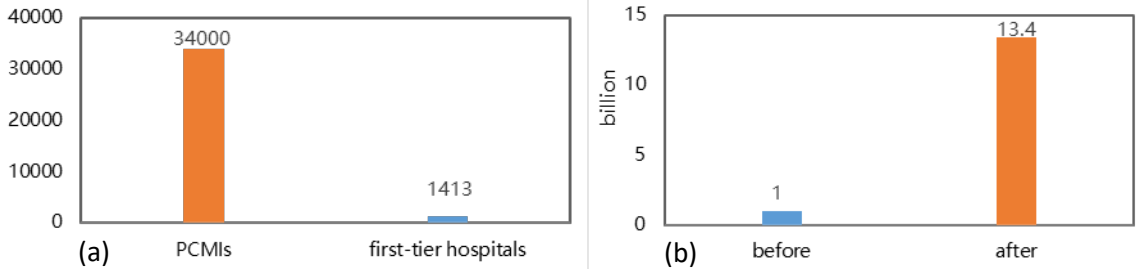


Fig. 11. (a) (2019) Number of primary-care medical institutions and first-tier hospitals in China; **(b)** POCT future growth space in the primary-care medical market of China before/after the policy implementation.

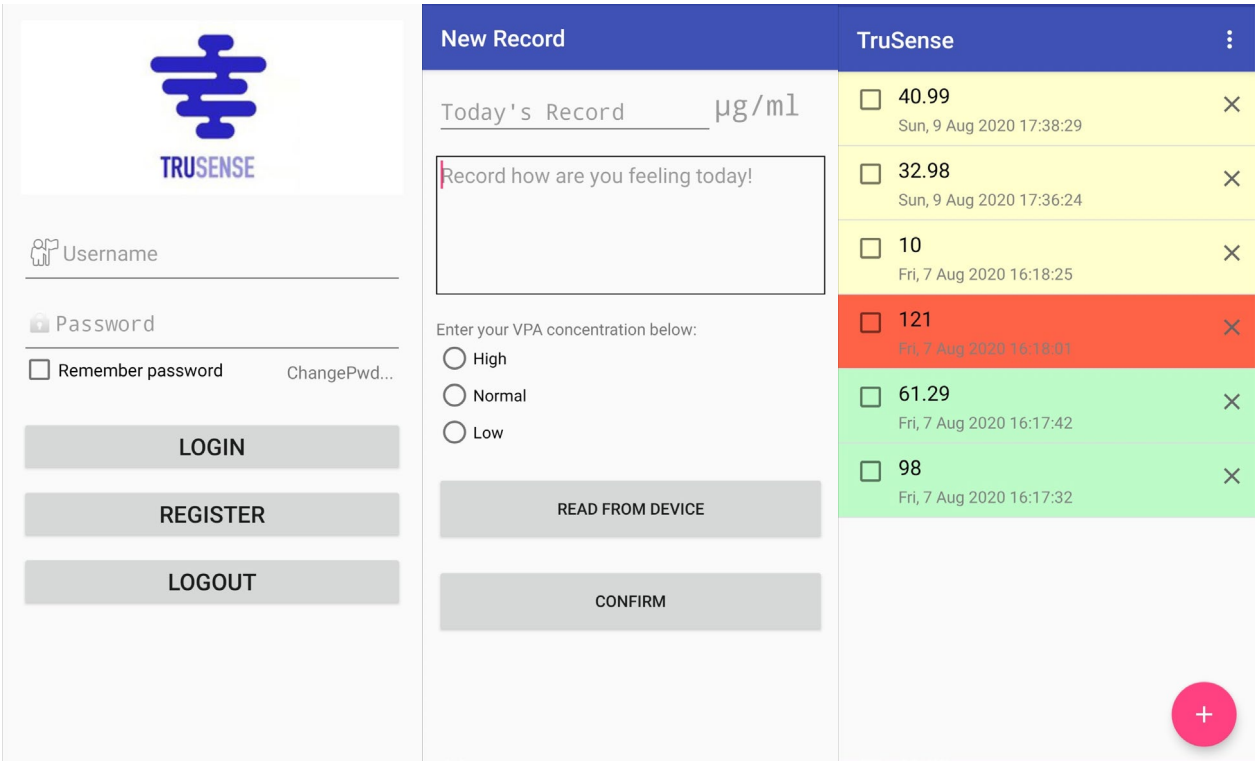


Fig. 12. User interface of our APP TruM.

TEAM RESULT DOCUMENT