Shark's olfactory system inspired mems-based and flexible LCP-based chemical sensors for heavy metal detection

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**SHARK'S OLFACTORY SYSTEM INSPIRED MEMS-
BASED AND FLEXIBLE LCP-BASED CHEMICAL
SENSORS FOR HEAVY METAL DETECTION**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING
2017**

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BASED AND FLEXIBLE LCP-BASED CHEMICAL
SENSORS FOR HEAVY METAL DETECTION**

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ABSTRACT

Water pollution has become an issue of serious concern on a global scale. Among different pollutants, heavy metal ions are considered as one of the most hazardous contaminants since they cannot be biologically degraded and will accumulate in the human body for a long period of time. Conventional water quality monitoring methods typically rely on collecting water samples in the field and then bringing them to localized laboratories for testing. However, there are some inevitable disadvantages such as labor-intensive sample collection, time-consuming preparation and analysis, and costly experimental apparatus. Real-time data regarding the level of target metal ions in concerned sites cannot be attained through the conventional approaches. Therefore, the development of miniaturized, inexpensive, and portable chemical sensors for on-site/in-situ heavy metal monitoring is highly desirable. In this thesis, lead (Pb) and zinc (Zn) are selected as target heavy metal ions in consideration of the historical situation of heavy metal contamination in Singapore. The environmental regulatory values for Pb and Zn ions in drinking water are 10 ppb ($\mu\text{g/L}$) and 3 ppm (mg/L).

For Pb ions detection, a miniaturized, lab-on-a-chip chemical sensor is designed by drawing inspiration from the morphological structure of shark's olfactory system. The sensor features a bottom sensor base with three thin-film, photolithographically-fabricated electrodes, and a top microfluidic channel. A three-dimensional, free-standing micropillar working electrode array is incorporated into the sensor, which mimics the configuration of shark's sensory cells. To the best of our knowledge, this is the first time that a shark's olfactory system inspired chemical sensor has been developed. The sensor was successfully fabricated by means of microelectromechanical systems (MEMS) techniques. The analytical performance of the biomimetic chemical sensor was comprehensively investigated through electrochemical experiments. Under optimal conditions, an analytical sensitivity of 32 nA/ $(\mu\text{g/L})$ and

favorable limit of detection (LOD) of 0.2 ppb were obtained under a short deposition time of 30 s. The sensor exhibited a linear response to Pb ions in the concentration range from 1 to 130 $\mu\text{g/L}$. It was found that the bio-inspired design significantly enhanced collection efficiency of the sensor towards target metal ions. This is both due to the enlargement of electrode surface area and the interaction between protruding micropillar working electrodes and moving solution. The proposed sensor eliminates the involvement of mechanical stirring of testing solution, manifesting great potential to be deployed for the application of on-site Pb ions determination.

Driven by the attempt to achieve in-situ Zn ions detection, a flexible chemical sensor integrated on a liquid crystal polymer (LCP) substrate is developed. Incorporation of the LCP material renders improved operational reliability, high durability, as well as extraordinary flexibility to the sensor, which allows it to be mounted on any flat or curved surface for harsh environmental sensing. The fabrication of the sensor was achieved through standard MEMS techniques. Experimental evaluation showed that the sensor was able to detect Zn ions down to 0.08 ppb. In addition, the sensor showed an analytical sensitivity of 92 nA/($\mu\text{g/L}$), gratifying detection range from 0.3 to 70 $\mu\text{g/L}$, and favorable reproducibility with relative standard deviation of 1.64% ($n = 6$). Real-time application of the sensor was also demonstrated by measuring Zn ions in seawater using a sensor array attached to the hull of a remotely-controlled autonomous kayak. The response of the sensor array indicated a clear trace of Zn ions present in the seawater, which was subsequently confirmed by performing inductively coupled plasma mass spectrometry analysis with collected seawater samples. The laboratory and field investigations suggest promising application of the proposed flexible chemical sensor for in-situ Zn determination.

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1

INTRODUCTION

This chapter presents a brief introduction of the background of the research topic, including current situation of global water pollution, different categories of water pollutants, and problems associated with conventional water quality monitoring approach. Driven by the concerns, objectives of this project are defined and elaborated in terms of concept and methodology.

1.1 Current situation of global water pollution

The importance of water is self-evident. It is considered to be one of the most indispensable prerequisites for human survival. However, over the last few decades, water resource scarcity, which has become an issue of global concern, brings tremendous adverse effects on the sustainable development of both human society and ecological system. The complexity and scope of water-related problems have stepped over national boundaries, suggesting the necessity of global communication and collaboration in terms of evaluating and tackling the challenges. In spite of the immense efforts made by public agencies and international communities, 20 percent of the world population, which is around 1.2 billion people, is still experiencing the shortage of drinkable water, as estimated by the International Water Management Institute (IWMI) [1]. Although nearly 70 percent of the Earth's surface is covered with water, unfortunately only about 3 percent of the total water volume can be considered as fresh water [2] with salinity less than 0.035% (which is about 0.35 grams of salts in 1 kilogram of water). Among such limited volume of precious fresh water, surface fresh water which is widely deemed as a renewable resource since it can be naturally recycled within certain watershed, accounts for merely 0.3 percentages [2], as shown in Figure 1.1 (a). Therefore, how to effectively distribute, govern and make use of surface water is extremely critical for us in order to maintain the harmonious relationship between human society and natural environment.

On the other hand, degradation of water quality which is mainly caused by uncontrolled population growth, excessive industrial expansion and immoderate anthropogenic activities especially discharge of untreated wastewater, further exacerbates the burden of water resource management, resulting in more threat to the safety of drinking water. It is reported that, in some developing countries, around 70 percent of industrial wastewater without any

pre-treatment is directly dumped into lakes and rivers [3], which are the two most primary storage places for surface fresh water [2], as shown in Figure 1.1(b). More worryingly, the extent of water pollution is not only happening at the level of surface water, but also sprawling towards the underground water in which almost 30 percent of the Earth's fresh water is preserved, as shown in Figure 1.1(a). Poor water quality can be a major threatening factor for the health of human beings as well as the ecological environment. Based on statistical data published by the World Health Organization (WHO), "24% of global disease burden and 23% of all deaths are attributed to environmental factors", within which about "94% of the diarrhoeal disease is associated with factors such as unsafe drinking water, poor sanitation, and hygiene" [4]. The consequence of water quality deterioration is not limited to health problems. In addition, dramatic increase in water treatment expenditure, severe shortage of drinkable water and huge financial loss due to unavailability of safe water for agricultural production and food processing are all associated with the rapid spread of water pollution.

Figure 1.1: (a) Constitution of the 3% fresh water [2]. (b) Distribution of the 0.3% surface fresh water [2].

1.2 Categories of water pollutants

There are different kinds of pollutants that could be carried into lakes and rivers by either surface water or underground water pathways. Dominating water pollutants can be roughly

categorized into four groups, namely macroscopic pollutants, pathogens, organic pollutants and inorganic pollutants. Macroscopic pollutants mainly refer to the garbage that is discarded by inconsiderate people or dumped rubbish that may be transported by the municipal drainage system. Pathogens like *cryptosporidium parvum*, *giardia lamblia*, norovirus, etc., are usually brought to water by contaminated excreta to induce chronic or acute waterborne diarrheal diseases. Organic contaminants such as detergents, insecticides, herbicides, and petroleum byproducts, upon being released to the watercourses, significantly increase the amount of dissolved oxygen needed in order to break down the organic material. The reduced oxygen level deteriorates the fitness or even causes the asphyxiation of living organisms. Besides, large quantities of organic substances limit the light available to the photosynthetic plants owing to the increased turbidity.

Inorganic pollutants are another major category of water pollution, among which heavy metal contaminants, which are the focus of this project, are believed to be more insidious and hazardous because of their long-term retention in the human body as well as high resistance to natural degradation. For instance, cadmium (Cd) is able to stay in the kidney for 20 or 30 years [5, 6], which will gradually impair the metabolism-related enzymes. Moreover, a number of other heavy metals, such as arsenic (As), lead (Pb), mercury (Hg), copper (Cu) and zinc (Zn), are extremely toxic under high concentration. Specifically, the kidney is the primary target of As and other health problems such as hypertension, cardiovascular disease, cardiomyopathy and toxic hepatitis may also be induced depending on the levels of exposure [7]. Superfluous Pb will restrain the formation of hemoglobin, which is one of an essential component of red blood cells [8]. Other signs such as headache, slow reaction, tremors and impaired renal excretion may also occur when the exposure concentration rises up to certain limit value. Adverse pathological effects of Hg include decreased acuity of sensory organs,

physical fatigue, numbness, anemia and autoimmune disorders [9]. In regard to Cu, pathological abnormalities are varied from gastroenteritis, hemolytic anemia, renal damage to transient hepatic dysfunction based on the level of intake [10]. Extensive quantity of zinc ingestion can induce severe gastrointestinal problems, hair loss, muscle spasms, sciatica, etc. [11].

1.3 Conventional water quality monitoring

Given the non-biodegradable nature along with the detrimental health effect for humans pertaining to heavy metal contamination, it is of foremost importance to perform continuous monitoring of the pollution status at different locations of crucial water areas. Conventional water quality monitoring, as shown in Figure 1.2, is typically carried out in the following procedures. In the beginning, water samples are collected from several fixed and/or random sites based on the types of pollution problems that may be encountered, after which packaged samples will be transported to the localized laboratories. Subsequently, comprehensive experiments are conducted by specialists for the purpose of providing detailed information regarding the overall quality of the water at specified regions. Both physical parameters including temperature, conductivity, turbidity, total dissolved solids, color and odor of water, and chemical parameters including pH value, dissolved oxygen, nutrients, heavy metals, and pesticides are used to indicate the water condition. Furthermore, biological indicators especially the so-called EPT (Ephemeroptera, Plecoptera, and Trichoptera) indexes [12] are also widely measured to represent the diversity of aquatic animals in the tested waters.

Figure 1.2: Procedures of conventional water quality monitoring.

However, traditional water surveillance method has many disadvantages that may partially or totally distort the testing results. In the first place, there are some unpredictable reactions that may occur in the samples since transportation always takes quite a long period of time. Any changes in the collected water samples could greatly affect the accuracy of the laboratory analysis. It is almost impossible to control or alleviate these reactions because experimental outcomes cannot be trusted if too much manual perturbation is involved. More importantly, collecting water samples is tremendously time-consuming and labour-intensive in that it is unavoidable to gather sufficient samples in different waterways. At certain places, it is even unattainable to collect the water. Another drawback of conventional water pollution monitoring is manifested by the issue that facilities used to carry out the laboratory analysis are restricted to predetermined location, let alone its costly expenditure and requirement for well-trained personnel to operate the equipment.

To circumvent the aforementioned issues, recent trend pertaining to water quality monitoring has progressed towards direct deployment of environmental sensors to the locations of interest in order to acquire real-time data. The emergence of the concept “Internet of Things

(IoT)” provides high possibility to achieve on-site/in-situ measurement of water quality. According to the Global Standards Initiative on Internet of Things, IoT is defined as “a global infrastructure for the information society, enabling advanced services by interconnecting (physical and virtual) things based on existing and evolving interoperable information and communication technologies” [13]. In other words, the IoT integrates the physical world with the virtual one, where a huge amount of data collected by different kinds of embedded sensors/devices from diverse locations, are stored, processed, and shared globally. Such advanced interconnection renders the IoT to be applied in almost all the fields, including manufacturing, transportation, urban planning, infrastructure management, environmental sensing, home automation etc. This future networking system greatly promotes a rapid development of IoT-based sensors and devices. With regard to water quality monitoring, some products, e.g. CENSAR MP from CENSAR Technologies Inc. [14], KAPTA 3000-AC4 from Veolia Water Technologies [15], and Intellisonde from Intellitect Water Ltd. [16], have been commercially developed. Although these sensors can only detect a few parameters, it is foreseeable that with a large-scale distribution of multiple types of sensors, in-situ tracking of water quality at locations of interest will be achieved.

1.4 Objectives of the project

Considering the complexity of conventional water quality monitoring, the development of compact, portable/disposable, sensitive, and inexpensive sensors, which can be employed in the IoT system, would be highly desirable. Therefore, this project is targeting on the development of novel chemical sensors by taking advantage of microelectromechanical systems (MEMS) fabrication technology for potential on-site/in-situ detection of heavy metal contamination. Instead of making conventional chemical sensors, the MEMS chemical sensor proposed in this project tries to get the inspiration from nature to mimic both morphological

and functional virtues of the olfactory sensing system of pelagic sharks, in such a way that minute variation of the concentration of heavy metals can be quickly detected. By employing the proposed cost-effective, miniaturized and sensitive MEMS chemical sensors, it is possible to obtain real-time information pertaining to the levels of target heavy metals in concerned water areas.

1.5 Merits of biomimetic approach

It is necessary to recall the objective of this project, which is to develop a kind of chemical sensor that can be employed for on-site and/or in-situ heavy metal evaluation without intricate operation and external intervention. The originality of this project lies in bringing the bio-inspired and biomimetic approach into the design of artificial chemical sensor to explore new possibilities. Nature always provides inspiration for human beings to explore its beauty and mystery. The survival instinct together with the rapid change of surrounding environment impels different creatures to be equipped with incredibly sensitive sensors. It is of great interest to develop artificial counterparts to mimic the high-performance biological sensors.

Pertaining to odorant sensing in water, the smelling capability of aquatic animals especially pelagic sharks is exceptionally famous. Biologists have reported that shark's olfactory sensing organs are heavily used for the acquisition and analysis of odorant information, which assists them in food searching, habitat recognition as well as reproduction [17]. Figure 1.3(a) depicts the slit-like nasal cavities of a tiger shark along one side of the snout, where interaction between odorant molecules and sensory receptors occurring in the olfactory organs, as shown in Figure 1.3(b), helps the shark in accurately locating the potential prey. Considering the performance indicators such as detection limit, response time, robustness, stability and so forth, biological sensing systems certainly surpass most man-made chemical

instruments. Therefore, the bio-mimicking approach will provide unlimited possibilities as well as innovative research directions for the development of artificial chemical sensors.

Figure 1.3: (a) Ventral view of a tiger shark (© Tomas Kotouc [18]) to show the slit-like nasal cavities along one side of the snout. (b) Schematic drawing to show the olfactory organ (OO) [19], where white triangular arrow illustrates flow direction through the olfactory sensing system.

1.6 Merits of MEMS technology

In order to achieve the abovementioned objectives, designing miniaturized, sensitive and robust chemical sensors with the aid of MEMS fabrication technology seems to be a promising and practical approach. MEMS technology has been proved to be incredibly successful in the field of microsystem engineering, which is almost related to all automatic sensing and actuating sensors in the defense, aerospace, automotive, biomedical, electronic and communication industries. The versatile application of MEMS technology is supported by certain attractive merits. To name a few, miniaturization of MEMS devices which are typically in the range of several micrometers to a few centimeters offers a lot of operational benefits such as higher resonance frequency, higher sensitivity, better linearity and wider dynamic range. In general, the measuring accuracy of macro-sized instruments will generate considerable drift when they are subjected to external disturbances, e.g. ambient condition change, increased noise level and abrupt vibration, whereas these unexpected perturbations have less impact on the signal obtained with MEMS devices. Moreover, seamless

combination of the micromechanical part with electronic circuitry at the wafer level produces integrated MEMS product with computing, processing and decision-making capabilities. In addition, fine three-dimensional (3D) features can be precisely controlled and manufactured in a repeatable way by means of standard MEMS techniques. Considering the goal of this project, the usage of MEMS technology will ensure batch fabrication of miniaturized chemical sensors with repeatable dimensions. Hence, discrepancies of performance among different sensors can be significantly minimized. Furthermore, the diversity of MEMS technology provides high feasibility to fabricate chemical sensors with a variety of complicated 3D structures.

1.7 Outline of the thesis

The thesis is arranged into seven chapters. Chapter 1 presents a brief introduction of the background of the research topic, including current situation of global water pollution, different categories of water pollutants, and problems associated with conventional water quality monitoring approach. Driven by the concerns, objectives of this project are defined and elaborated in terms of concept and methodology.

Chapter 2 firstly specifies the target heavy metal ions of concern in this project by reviewing the historical literature regarding the situation of heavy metal contamination in Singapore. Subsequently, both advantages and limitations of different heavy metal detection methods are described in order to evaluate their feasibility for this project. After a comprehensive comparison, the electrochemical method, i.e. anodic stripping voltammetry, is selected and the reasons for such selection are justified. Finally, a number of valuable researches in the literature pertaining to some critical developments and major achievements employing the

anodic stripping voltammetry are reviewed, from which current challenge/demand is extracted.

Chapter 3 starts with a detailed description of shark's olfactory sensing system, which is the source of inspiration for this project. Thereafter, the structure, dimension, and sensing principle of a shark-inspired chemical sensor are presented. Some critical aspects that have been taken into consideration when the sensor is designed and some hypotheses/merits that could be acquired through the biomimetic approach are elaborated. Finally, detailed fabrication procedures and essential fabrication parameters of the biomimetic chemical sensor are reported.

Chapter 4 mainly focuses on the characterization of the on-chip reference electrode as well as the bio-inspired micropillar electrode array. Two types of on-chip reference electrodes, one of which is made of a layer of sputtered Ag and the other is made of evaporated AgCl coated on a sputtered Ag layer, are investigated in the aspects of a) stability over a range of scan rates, b) stability over a prolonged period of measurement, and c) response consistency over multiple reference electrodes. As for the bio-inspired micropillar electrode array, electrical connection, surface property, electrochemical sensing ability, and total Au coverage area are separately evaluated.

Chapter 5 provides a comprehensive assessment of the biomimetic chemical sensor towards lead detection. Parameters such as the potential window, deposition potential, and deposition time, which will affect the performance of the sensor, are studied. In particular, the effect of flow rate on the biomimetic chemical sensor without and with micropillar electrode array is inspected. The possible reasons that could induce the interesting experimental observations

are explained, through which the proposed hypotheses are verified. The last section presented in this chapter documents the analytical performance of the biomimetic chemical sensor, including the sensitivity, linear range as well as limit of detection.

Chapter 6 details out the design of a miniaturized and compact chemical sensor for zinc detection constructed on a novel robust material, liquid-crystal polymer (LCP). Incorporation of the LCP substrate offers improved reliability, high durability and flexibility to the sensor, rendering it surface-mountable feature for in-situ deployment. The fabrication procedures of the sensor are provided and sensing capability of the sensor is optimized in aspects of the potential window, deposition potential, and deposition time. Reproducibility, interference, and analytical performance of the sensor are also investigated. Real-time applicability of the sensor is corroborated by performing sea trial testing with a sensor array attached to the hull of a remotely-controlled kayak.

Chapter 7 firstly presents the development of an MEMS-based aptasensor for norovirus detection. The aptasensor is built by modifying the biomimetic chemical sensor without micropillar electrode array with biological sensing elements, i.e. norovirus-specific aptamers. The detailed fabrication, visual and electrochemical confirmation of aptamer immobilization, and analytical performance towards murine norovirus determination of the sensor are provided. Thereafter, conclusions and major contributions of this project are summarized. The last section of this chapter points out some possible improvements pertaining to this research topic for future work.

References

- [1] Cooley, H., Ajami, N., & Ha, M. L. et al. (2014). Global water governance in the twenty-first century. In P. H. Gleick, *The world's water* (pp. 1-18). Boca Raton, FL: CRC Press.
- [2] Bralower, T., & Bice, D. (2017). Distribution of water on the Earth's surface. Retrieved July 6, 2017, from <https://www.e-education.psu.edu/earth103/node/701>.
- [3] UN-water: Statistics. (2016). In *UN-Water*. Retrieved from <http://www.unwater.org/statistics/statistics-detail/en/c/211800/>.
- [4] Pruss-Ustun, A., & Corvalan, C. (2006). *Preventing disease through healthy environments: Towards an estimate of the environmental burden of disease* (ISBN: 9241593822). France: World Health Organization.
- [5] Lyon, T. D. B., Aughey, E., & Scott, R. et al. (1999). Cadmium concentrations in human kidney in the UK: 1978-1993. *Journal of Environmental Monitoring*, 1(3), 227-231.
- [6] Satarug, S., Baker, J. R., & Reilly, P. E. B. et al. (2002). Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. *Archives of Environmental Health*, 57(1), 69-77.
- [7] Jomova, K., Jenisova, Z., & Feszterova, M. et al. (2011). Arsenic: toxicity, oxidative stress and human disease. *Journal of Applied Toxicology*, 31(2), 95-107.
- [8] Counter, S. A., Buchanan, L. H., & Ortega, F. (2012). Association of hemoglobin levels and brainstem auditory evoked responses in lead-exposed children. *Clinical Biochemistry*, 45(15), 1197-1201.
- [9] Houston, M. C. (2011). Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. *The Journal of Clinical Hypertension*, 13(8), 621-627.
- [10] Barceloux, D. G. (1999). Copper. *Clinical Toxicology*, 37(2), 217-230.

- [11] Roohani, N., Hurrell, R., & Kelishadi, R. et al. (2013). Zinc and its importance for human health: an integrative review. *Journal of Research in Medical Sciences*, 18(2), 144-157.
- [12] Stoyanova, T., Vidinova, Y., & Yaneva, I. et al. (2014). Ephemeroptera, Plecoptera and Trichoptera as indicators for ecological quality of the Luda Reka River, Southwest Bulgaria. *Acta Zoologica Bulgarica*, 66(2), 255-260.
- [13] International Telecommunication Union. (2017). Internet of Things Global Standards Initiative. Retrieved July 7, 2017, from <http://www.itu.int/en/ITU-T/gsi/iot/Pages/default.aspx>.
- [14] CENSAR Technologies Incorporated. (2009). Catalog. Retrieved July 7, 2017, from <http://www.censar.com/html/catalog.html>.
- [15] Veolia Water Technologies. (2014). KAPTA™, in-line multi-parameter water sensors. Retrieved July 7, 2017, from <http://www.endetec.com/en/products/kapta/>.
- [16] Intellitect Water Limited. (2015). This is the Intellisonde. Retrieved July 7, 2017, from <http://www.intellitect-water.co.uk/our-product>.
- [17] Hueter, R. E., Mann, D. A., & Maruska, K. P. et al. (2004). Sensory biology of elasmobranchs. In J. C. Carrier, J. A. Musick, and Michael R. Heithaus, *Biology of sharks and their relatives* (pp. 325-368). Boca Raton, FL: CRC Press.
- [18] Tiger shark, ventral surface close up. (n.d.). [Photograph]. Retrieved October 1, 2016, from <http://www.arkive.org/tiger-shark/galeocerdo-cuvier/image-G141472.html>.
- [19] Cox, J. P. L. (2013). Ciliary function in the olfactory organs of sharks and rays. *Fish and Fisheries*, 14(3), 364-390.

2

LITERATURE REVIEW

This chapter firstly specifies the target heavy metal ions of concern in this project by reviewing the historical literature regarding the situation of heavy metal contamination in Singapore. Subsequently, both advantages and limitations of different heavy metal detection methods are described in order to evaluate their feasibility for this project. After a comprehensive comparison, the electrochemical method, i.e. anodic stripping voltammetry, is selected and the reasons for such selection are justified. Finally, a number of valuable researches in the literature pertaining to some critical developments and major achievements employing the anodic stripping voltammetry are reviewed, from which current challenge/demand is extracted.

2.1 Target heavy metal ions

In order to determine the heavy metals that should be targeted in this project, historical literature regarding the situation of heavy metal contamination in Singapore has been thoroughly reviewed in the chronological order. Back to the middle of 20th century, the water in the Singapore River was heavily polluted because of the indiscriminate discharge of wastes and rubbish, which made the water almost impossible for aquatic lives to survive. Attempting to completely remedy such terrible condition, in 1977, a 10-year-programme was launched by Singapore government to improve the quality of the water. Eleven years later, Sin et al. [1] led a research group to collect the water samples, sediment, mud, fishes, and crabs at 5 stations along Singapore River from the Anderson Bridge down to the Kim Seng Bridge within 6 months. They analyzed heavy metal accumulation within the flesh of fishes and crabs by using atomic absorption spectrophotometer. Results showed that water quality was significantly improved as dissolved oxygen levels increased to 3 mg/L compared with almost 0 mg/L 10 years ago. However, examination of sediment samples indicated that the degree of heavy metal pollution in the middle part of the Singapore River, which was a boat quay, was much higher than other stations with the proof of increased amounts of Cu, Cd, Zn, iron (Fe), and Pb in particular. Such observation could be explained by the fact that a lot of boats were powered by lead-containing fuel at that time, which might discharge or leach Pb into the river. The authors also measured the concentration of heavy metals in aquatic animals and found that bioaccumulation of Hg, Cd and Cu was quite obvious in the body of polychaetes, prawns, and crabs, suggesting heavy metal pollution could impose a huge threat to biota as well.

Another thorough investigation about heavy metal contamination in the marine environment of Singapore was conducted by Goh and Chou [2] from December 1990 to July 1992. They selected 20 locations representing different ecosystems along the coastal line of Singapore to

routinely test the sediment samples every three months. All the preparation and examination procedures were performed by strictly following the standard protocol to minimize the measurement errors. After digesting the samples by 65% nitric acid along with dissolving in 10% nitric acid twice, sediment solutions were evaluated by means of the atomic absorption spectrophotometer. Subsequently, the data were further treated with the Multidimensional Scaling (MDS) method to check the statistical significance. Results clearly showed that Cu, Pb, Cd and Zn were the main pollution sources for the Singapore's coastal water during that period. They also reported that the concentration level of heavy metals was related to sediment particle size, implying that heavy metals were prone to stick on finer sediments due to the larger surface areas provided. The authors speculated that the possible reason for Cu and Zn pollution could be the consequence of anti-fouling paint leakage from transporting ferries.

Stepping into the 21st century, Bayen and his colleagues [3] chose 8 stations around the offshore areas of the Singapore's main island between April and May 2002 to collect 20 pieces of *Perna viridis* green mussels for assessing biologically accumulated levels of organochlorine pesticides and heavy metals, since mussels can be regarded as a kind of bio-indicator to show the extent of environmental pollution. By digesting mussel tissue with 30% hydrogen peroxide and 65% nitric acid, samples were studied to determine the concentrations of As, Cu, Pb, Cd, chromium (Cr), nickel (Ni) and Zn. Although experimental outcome indicated that the tissue of green mussel had all heavy metals listed above, only As, Cu, Pb, and Zn were approaching the safety limiting values. A recent research carried out by Yuen et al. [4] in 2012, came to the similar conclusion that the concentrations of Cu, Pb and Zn exceeded safe levels for aquatic organisms. Researchers collected road deposited sediments (RDS) samples from 15 residential and 15 industrial road sites covering almost the whole

main island of Singapore to quantify the contaminant condition of eleven elements including aluminium (Al), cobalt (Co), Cr, Cu, Fe, Ni, Pb, antimony (Sb), scandium (Sc), silicon (Si) and Zn. Toxic elements in RDS can be easily accumulated since they have fine structure and readily transported to nearby waters by strong wind or overland flow if street sweeping doesn't efficiently clean the RDS away. Under a stringent preparation scheme, the RDS samples with grain size less than 63 μm were examined by three methods, including a) comparison with the consensus-based threshold effect concentrations (TEC) and portable effect concentrations (PEC), b) calculation of the enrichment ratio (ER) if no TEC and PEC values were available and c) evaluation of the Tomlinson multi-element pollution load index (PLI). Their results showed that Cu, Pb, Sb and Zn were significantly enriched in both industrial and residential areas, where Cu, Pb, and Zn were beyond the established safety guidelines.

From the past literature, it is possible to infer that Cu, Pb, and Zn heavy metal ions are the three types of the most prevalent contaminants in Singapore, which will be the focus of this project. Referring to the drinking water quality guidelines published by the WHO, recommended values for Cu, Pb and Zn heavy metal ions in drinking water are 2 mg/L, 10 $\mu\text{g/L}$ and 3 mg/L [5], respectively. Among these three pollutants, Pb is supposed to be the most toxic element not only because its threshold number is nearly 200 times lower than the other two elements, but also because it is relatively abundant in the workplace as well as the dwelling houses. A lot of human organs and tissues such as heart, kidneys, lungs, bone and even nervous system are susceptible to the increased level of Pb [6, 7]. Furthermore, especially for children, Pb can bring about permanent learning and behavior disorders [7]. Therefore, we will start the project by targeting on the Pb detection. Once the sensing

capability of the proposed MEMS chemical sensor is verified, other kinds of heavy metal ions can also be attempted.

2.2 Heavy metal detection methods

2.2.1 Inductively coupled plasma mass spectrometry

There are many approaches available for measuring the concentration of different metal ions. Inductively coupled plasma mass spectrometry (ICP-MS) is arguably one of the most commonly used commercial techniques worldwide for trace element analysis. Application of ICP-MS covers a variety of research fields, including semiconductor, metallurgy, geochemistry, biopharmaceutics, clinical examination, analytical chemistry, environmental science, nuclear technology and so forth. A brief schematic illustration of the ICP-MS is shown in Figure 2.1, where the argon (Ar) gas is introduced into the torch, which is powered by a radio-frequency (RF) generator. When an electric spark is applied to the Ar stream, free electrons are brought into the torch and then they interact with the oscillating electric and magnetic fields to collide with Ar atoms, forming a high-energy plasma. The sample is usually introduced into the plasma in the form of aerosol with the aid of a nebulizer and a spray chamber in order to evaporate the solvent of the sample. Under the influence of the high-temperature plasma, the constituent atoms of the sample are almost instantaneously ionized. Thereafter, the ions produced are sampled through the interface cones (sampler and skimmer cones with small orifice) and be focused into the quadrupole mass spectrometer by a series of electrostatic ion lenses. Finally, an ion detector is used to convert the ions to the electrical signal, which is proportional to the concentration of the analyte ions. Determination of Cu [8-10], Pb [8, 11-13] and Zn [8, 14-16] in different specimens using the ICP-MS method has been widely reported. However, owing to the involvement of several separate

components combined with its complexity of the assembly, it seems that this method is not suitable for making miniaturized heavy metal detector.

Figure 2.1: Schematic illustration of the inductively coupled plasma mass spectrometry method [17].

2.2.2 Surface plasmon resonance

Over the last few decades, a number of researchers have devoted countless time and effort to develop simple, inexpensive and sensitive methods for heavy metal measurement. These methods, from the functionality point of view, are supposed to be comparable to the conventional commercially-available approach, e.g. the aforementioned ICP-MS method, and be compatible with the design, fabrication, and assembly of micro-sized sensors. Three of them will be presented herein to seek out the most suitable alternative. The first method is called surface plasmon resonance (SPR), which detects the difference of the resonant condition of a metallic film before and after analyte binding. Figure 2.2 sketches the setup of a differential SPR, proposed by Zhang et al. [18, 19]. It is based on the widely known Kretschmann configuration, in which a p-polarized laser beam is focused onto a piece of gold (Au) film (placed on a prism) with two sections, i.e. the sensing area and the reference area. The incident beam will then be adsorbed by the surface plasmon at the resonance angle, leading to minimum reflection. Under such condition, a dark line (SPR dip) which is correlated with the resonance of the surface plasmon can be observed.

Figure 2.2: Schematic illustration of the differential surface plasmon resonance method [19].

As shown in Figure 2.3, a quadrant cell photodetector is responsible for recording the SPR dips from both sensing (A, B) and reference (C, D) areas at the same time. Calibration of the photodetector is required, ahead of each measurement, to guarantee that the signal denoted by $(A-B)/(A+B)$ and $(C-D)/(C+D)$ is in the equilibrium position. The function of the peptides implanted on the sensing area is to specifically capture target metal ions. While employing dodecanethiol (DDT) monolayer turns the surface property of the reference area from hydrophilicity to hydrophobicity. The resonant difference between the reference and sensing areas is related to the corresponding SPR dip locations, from which the amount of adsorbed metal ions can be calculated. Researchers have reported the usage of the SPR method to quantify the level of Cu [20, 21], Pb [22, 23] and Zn [24] ions using different recognition molecules. The simplicity of the setup is a distinct advantage for this method, but miniaturization of optical configuration is not easy to be incorporated into the process of micro-fabrication.

Figure 2.3: Sensing principle of the differential surface plasmon resonance method [19].

2.2.3 Ion-selective electrode

The second of the simple and inexpensive methods involves the employment of the ion-selective electrode (ISE), which consists of an ion-selective thin membrane that only allows certain kind of ions to be transported. As shown in Figure 2.4, an ISE has a body/container which is filled by the internal electrolyte solution, within which an internal reference electrode, normally a silver/silver chloride (Ag/AgCl) electrode, is immersed. The movement of the target ions across the membrane will generate a potential difference with respect to the external reference electrode, which can be measured by a voltmeter. The potential value obtained is proportional to the concentration of target ions in the solution. The ISE device can be treated as a chemical sensor, whose function is to transfer the chemical information into an analytical, or more commonly, electrical signal.

Figure 2.4: Schematic illustration of the ion-selective electrode [25].

In fact, a lot of potentiometric ISE sensors that are capable of detecting toxic heavy metal ions have been extensively presented over the last fifteen years. By doping the membrane with different materials, detection of Cu [26-29], Pb [30-33] and Zn [34-37] has been achieved with wide detection range and relatively low detection limit. However, considering the on-site/in-situ applicability of these ISE sensors, there are still several limitations: 1) operational stability of ISE sensors is not good in that the electrodes themselves are too fragile to be deployed on the movable platform/vehicle for field measurement; 2) ability of ISE sensors to be miniaturized is considerably inhibited by the doping procedure into the ion selective membrane; 3) robustness of ISE sensors is not satisfactory since the membrane can be easily blocked or contaminated by proteins or other organic substances in the solution; 4) measurement uncertainty of ISE sensors in real samples could be very high as ligands and unknown ions with similar activity may interfere the response of the sensors.

2.2.4 Anodic stripping voltammetry

The last of the simple and inexpensive methods is known as anodic stripping voltammetry (ASV), which is an electroanalytical technique to quantitatively determine the concentration of ionic species. In recent years, devices based on electrochemistry have become promising measuring tools in the assessment of environmental pollution. Electroanalytical methods are often used to investigate the analyte of interest inside an electrochemical cell by either controlling or measuring the potential or the current. There are two types of methods, i.e. static techniques including potentiometry and dynamic techniques including coulometry, amperometry, and voltammetry. Potentiometry is to measure the potential of the electrochemical cell with respect to time when almost zero current passes through the solution of the analyte. Coulometry is to measure the current of the electrochemical cell with respect to time by providing either constant potential or constant current. Amperometry is to measure the current of the electrochemical cell as a function of fixed potential. Voltammetry is to measure the current of the electrochemical cell as a function of varied potential. The ASV is one subclass of voltammetry to continuously monitor the current when the potential is changed with respect to time. In general, the ASV is comprised of three electrodes: a working electrode, a reference electrode, and an auxiliary/counter electrode, as shown in Figure 2.5(a). During the measurement, the potential is applied to the working electrode with respect to the reference electrode, where the current is recorded between the working electrode and the counter electrode. All electrochemical reduction and/or oxidation reactions will be precisely controlled at the working electrode. Prior to the experiment, the testing solution is usually purged by nitrogen gas through a glass tube, as shown in Figure 2.5(a), in order to remove the dissolved oxygen.

The whole experimental process can be described in four steps, which is graphically illustrated in Figure 2.5(b). In the beginning (step A), a potential that is relatively more positive than the oxidation potential of the analyte is applied to the working electrode for a certain period of time to clean the electrode surface. The potential and the time in this step are called conditioning potential and conditioning time, respectively. Thereafter (step B), the potential is shifted to a more negative one than the oxidation potential of the analyte to initiate the deposition of the metal ions to the working electrode. This potential, which is called deposition potential, is also held for a period of time, which is called deposition time. Usually, stirring of the testing solution with the help of a magnetic stir bar, as shown in Figure 2.5(a), is provided in both step A and step B to enhance the cleaning efficiency and to increase the amount of analyte to be deposited on the working electrode surface, respectively. Followed by the deposition, the stirring is stopped to make the solution to be quiescent (step C), a period of which is called quiescent time. Subsequently, the potential is swept from a more negative to a more positive direction in a linear, staircase, differential pulse or square wave manner (step D). During this step, the previously deposited metal layer will be oxidized to the ionic state and stripped off from the working electrode. A peak or few peaks if the solution contains multiple ions will be observed in the current-potential curve, which is normally termed as voltammogram, in which the peak potential is corresponding to the stripping/oxidation potential of the analyte. Meanwhile, the magnitude of the current will indicate the concentration of the metal ions that are present in the testing solution. It is necessary to point out that the graphical depiction recommended by the International Union of Pure and Applied Chemistry (IUPAC) will be adopted to all the voltammograms presented in this thesis, where more positive potentials are plotted to the right on the x-axis and the anodic currents measured when the oxidation of the analyte happens are treated as positive currents plotted upwards on the y-axis.

Figure 2.5: (a) Schematic illustration of the electrochemical cell configuration [38] used in the anodic stripping voltammetry method. (b) Sensing process of the anodic stripping voltammetry [39], where A, cleaning step; B, deposition step; C, quiescent step; D, stripping step.

2.3 Selection of detection method for the project

The ASV approach is finally selected as the detection method in this project for the reason that electrochemical stripping analysis is considered, by a lot of researchers, as one of the most accurate, convenient and powerful techniques for simultaneous measurement of multiple heavy metal ions. The remarkable sensitivity arises from the contribution of the productive deposition step, which ensures sufficient amount of the analyte of interest to be accumulated, thereby making the detection of trace level of metal ions possible. Another beneficial point of the ASV method is that the electrode configuration can be oriented to take the advantage of MEMS manufacturing technology. It means that the fabrication of solid-state thin-film electrodes with different geometries can be easily achieved by using classical MEMS processes, including microstructure photolithographic patterning, thin film deposition, etching, etc. In addition, the whole setup of the ASV method is very simple in which only conductive electrodes are required. As a result, it is highly possible to scale down the overall dimension of the chemical sensor. In other words, miniaturization of both sensing elements

and accessory components is readily achievable without extensively compromising the performance of the chemical sensor.

2.4 Latest developments of anodic stripping voltammetry

For the ASV technique, the choice of an appropriate working electrode is extremely vital since the surface of the working electrode is the place where redox reaction of target metal ions occurs. An ideal working electrode should offer a completely renewable surface for more kinds of metal ions to be accumulated on it during the deposition step and produce low background noise to the stripping signal. At first, hanging mercury drop electrode (HMDE) [40-43] and mercury film electrode (MFE) [44-47] are widely accepted on account of the fact that plenty of heavy metals are prone to form an alloy with mercury (Hg), which is usually called amalgam, resulting in attractive stripping performance. However, the toxicity of Hg makes the handling and disposal operations more complex in accompany with the concern of detrimental effect of releasing mercury to the environment. Furthermore, the use of Hg material is severely limited or prohibited by local laws and regulations in a lot of countries, leaving a situation that it is improper to designate Hg as the working electrode for on-site/in-situ heavy metal monitoring. Therefore, the exploration of non-Hg working electrode becomes a challenging topic in the beginning of 21st century.

2.4.1 In-situ plated bismuth film electrodes

In 2000, a research group led by Joseph Wang introduced the bismuth-coated carbon electrode [48] to replace the conventional HMDE and MFE. Compared with Hg, bismuth (Bi) is an environmentally friendly element which has very low toxicity. Therefore, Bi is allowed to be used in many life-related industries, such as pharmaceutical manufacturing, cosmetic additives as well as pigmentation. By simultaneously depositing bismuth and target metal

ions onto a glassy carbon supporting electrode, they proved that bismuth film electrode (BFE) could be an alternative non-Hg electrode material for stripping voltammetric measurement since well-defined reproducible response and high signal-to-background ratio were observed. They also obtained a wide accessible potential window from -1.2 to -0.2 V with respect to the Ag/AgCl (3 mol/L sodium chloride (NaCl)) reference electrode for the BFE, allowing the quantitative evaluation of five to six kinds of heavy metal ions. Their pioneering work opened the possibility of adopting BFE for both centralized and decentralized measurement of heavy metal pollution. After that, tremendous research has been conducted to investigate the voltammetric performance of Bi-involved electrode in the electroanalytical field.

The in-situ plated BFE attracts a lot of attention for research, in which the Bi ions are co-deposited on the working electrode together with the heavy metal ions of interest. Advantages of using in-situ plated BFE are manifested by the features like reproducible stripping signal, wide separation of neighboring peaks, broad linear detection range, and high signal-to-background ratio during the potentiometric stripping analysis [48]. In contrast to the amalgam formed during the deposition process for MFE, Bi forms low-temperature multicomponent alloys with different heavy metals, which contributes to the accumulation of metal ions on the working electrode. Hocevar et al. [49] investigated the behavior of an in-situ plated BFE, along with an Ag/AgCl (3 mol/L NaCl) reference electrode and a platinum (Pt) wire counter electrode, under the condition of constant current potentiometric stripping mode. The electrode showed unambiguous signals for measurement of Pb, Cd, and Zn metal ions. Under a deposition time of 300 seconds (s) with stirring, they observed limit of detection (LOD) of 0.8 and 0.2 $\mu\text{g/L}$ for Pb and Cd, respectively. Kefala et al. [50] reported a study to simultaneously examine Pb, Cd, and Zn by a well-known method, square wave anodic stripping voltammetry (SWASV), with an in-situ plated BFE on a rotating glassy carbon

supporting electrode, an aqueous Ag/AgCl reference electrode, and a Pt counter electrode. By scanning the potential from -1.4 to 0 V with respect to the Ag/AgCl reference electrode, they obtained LODs of 0.2, 0.2 and 0.7 $\mu\text{g/L}$ for Pb, Cd, and Zn, respectively, under a deposition time of 10 minutes (min) with stirring. In addition, de-Carvalho et al. [51] conducted a comprehensive experiment to compare the electroanalytical performance between HMDE, MFE and BFE for simultaneous determination of Pb, Cd, Cu and thallium (Tl) in highly saline solutions (seawater and dialysis concentrate samples) with an Ag/AgCl (3 mol/L potassium chloride (KCl)) reference electrode and a glassy carbon tip counter electrode. They found that interference effect of Pb and Tl stripping signals was quite pronounced when HMDE was used. Meanwhile, similar interference phenomenon was observed when BFE was chosen to simultaneously detect Bi and Cu. In seawater, the LODs for Pb, Cd, and Tl using MFE and BFE were 0.1 and 0.06, 0.05 and 0.043, and 0.70 and 5.10 $\mu\text{g/L}$, respectively under a deposition time of 60 s with 2000 revolutions per minute (rpm) stirring. The LODs calculated for these three metal ions in the dialysis concentrate samples were slightly higher than that of the seawater for both MFE and BFE. It can be noticed that BFE displayed comparable stripping performance to the MFE in terms of metal ion quantification.

On the other hand, there are a number of valuable academic papers dealing with the characterization of some important parameters which may affect the response of in-situ plated BFE. For example, Baldrianova et al. [52] carried out a thorough study to evaluate the effect of Bi concentration, which covered the range from 10^{-7} to 10^{-4} mol/L, on the stripping response of Pb and Cd metal ions with an in-situ plated BFE on both carbon paste and Au supporting electrodes, an Ag/AgCl (3 mol/L NaCl) reference electrode and a Pt coil counter electrode. They claimed that bismuth-to-metal ion concentration ratio did have a great impact on the measurement sensitivity of SWASV. However, in contrast to the conventional

statement that the concentration of mercury ions should be set at least 10 times higher than the one of target metal ions for in-situ plated MFE, the ratio less than 10 was also applicable for in-situ plated BFE. By conducting the experiment with small and moderate Bi ion concentration, they achieved satisfactory LOD of 0.1 $\mu\text{g/L}$ for Pb and 0.15 $\mu\text{g/L}$ for Cd under a deposition time of 120 s with stirring. In 2011, Baldrianova et al. [53] did another research to examine the influence of acetate concentration, pH value of the solution and conductivity of the solution on the stripping peak height of Pb and Cd for an in-situ plated BFE on a carbon microdisc supporting electrode with a saturated calomel reference electrode and a Pt coil counter electrode. They observed that stripping signal was improved in the condition that no acetate was added to the testing solution and the pH value of the solution was in the acidic region. According to their explanation, such phenomenon could be attributed to the fact that under such conditions, more metal ions would be in the free instead of complex form in the solution, making both deposition and stripping of the metal ions fast and easy.

2.4.2 Bismuth film electrodes modified on different supporting electrodes

A variety of chemically modified BFE with different supporting electrodes instead of the bare glassy carbon electrode and carbon paste electrode have been widely reported. To list a few, Kruusma et al. [54] investigated the performance of an in-situ plated BFE modified on the boron-doped diamond supporting electrode for Pb detection in diluted human blood samples with an Ag/AgCl (3 mol/L KCl) reference electrode and a Pt wire counter electrode. The boron-doped diamond electrode has the advantage of low background current as well as wide potential window. They obtained LODs of 19, 7.3 and 2.3 $\mu\text{g/L}$ under deposition times of 60, 120 and 300 s in the quiescent condition, respectively. By sonicating the solution with the aid of an ultrasonic generator during the deposition step, they found that the LODs were further improved to 5.4, 0.85 and 0.18 $\mu\text{g/L}$ under deposition times of 60, 120 and 300 s, respectively.

Cesarino et al. [55] characterized a Nafion-coated in-situ plated BFE modified on a graphite-polyurethane composite supporting electrode for simultaneous determination of Cu, Pb, Cd, Zn and Hg metal ions with a saturated calomel reference electrode and a Pt wire counter electrode. The graphite-polyurethane composite electrode exhibits some favorable properties, such as resistance to swelling in the aqueous media due to the hydrophobic nature provided by the polyurethane, low toxicity to the environment and easy preparation procedures. Under a deposition time of 120 s, they achieved the LODs of 0.17, 0.23, 0.25, 0.04 and 0.5 $\mu\text{g/L}$ for Cu, Pb, Cd, Zn, and Hg, respectively. The usage of graphene as the supporting electrode is also attractive since such two-dimensional single layer of carbon atoms offers fast electron mobility, large surface area, and high chemical reactivity. Wang et al. [56] adopted the solid-state carbon diffusion method to fabricate a graphene film supporting electrode, which was subsequently coated by a polyaniline layer and plated with Bi film. The modified electrode together with an Ag/AgCl (saturated KCl) reference electrode and a platinum mesh counter electrode was used to quantify Pb and Cd metal ions. The LOD of 0.07 $\mu\text{g/L}$ for Pb was obtained under a deposition time of 180 s with 400 rpm stirring. Other kinds of supporting electrodes, such as graphite-epoxy composite electrode [57], edge plane pyrolytic graphite electrode [58], and zeolite doped carbon paste electrode [59], are also reported.

2.4.3 Bismuth film electrodes modified with different materials

Instead of changing the supporting electrode, modification of BFE with a variety of materials especially polymers is another interesting research direction and has been extensively investigated. One of the most studied polymers used to alter the BFE is Nafion. Nafion is a sulfonated tetrafluoroethylene based polymer, which is chemically inert, non-electroactive, and hydrophilic. The cation-exchange ability of Nafion renders it to attract the cationic target metal ions and simultaneously to inhibit the movement of anions near the surface of the

working electrode. Jia et al. [60] chose two ion-exchange polymers, namely Nafion and poly(sodium 4-styrenesulfonate), to modify the BFE on a glassy carbon supporting electrode for trace detection of Pb and Cd with an Ag/AgCl electrode (saturated KCl) reference electrode and a Pt foil counter electrode. They characterized the modified electrode with the well-established $\text{Ru}(\text{NH}_3)_6^{3+/2+}$ redox pair and observed that these two polymers were capable of effectively improving the preconcentration of the $\text{Ru}(\text{NH}_3)_6^{3+/2+}$. They acquired LODs of 0.53 and 0.38 $\mu\text{g/L}$ for Pb and Cd under a deposition time of 120 s with stirring. Torma et al. [61] evaluated the analytical performance of a Nafion[®]/2,2'-bipyridyl-modified BFE on a glassy carbon supporting electrode towards the voltammetric analysis of Pb, Cd and Zn with an Ag/AgCl (3 mol/L KCl) reference electrode and a platinum wire counter electrode. They found that addition of Nafion helped the stabilization of the Bi film formed on the supporting electrode. Moreover, the chelating agent, 2,2'-bipyridyl, contributed to the enhancement of both sensitivity and selectivity of the measurement. They were able to obtain LODs of 0.08, 0.12 and 0.56 $\mu\text{g/L}$ for Pb, Cd, and Zn under a deposition time of 120 s with stirring. Jiang et al. [62] introduced a Nafion-coated BFE modified with an ionophore, N,N,N',N'-tetrabutyl-3,6-dioxaoctanedi(thioamide), on a glassy carbon supporting electrode for Cd quantification with an Ag/AgCl (3 mol/L KCl) reference electrode and a Pt counter electrode. They observed significantly increased stripping peak current for the hybrid electrode, which was highly possible due to the ion-exchange ability of the Nafion and the specific complexation of the ionophore to Cd ions. Under a deposition time of 180 s with stirring, they obtained promising LOD of 0.01 $\mu\text{g/L}$ for Cd and high selectivity over Cu, Pb, and indium (In). Other types of materials used to build modified BFE include Au nanoparticle-graphene-cysteine composite [63], electroreduced graphene oxide-supported thiolated thionine [64], electrochemically deposited graphene [65], etc.

2.4.4 Screen-printed electrodes

Although the LOD of heavy metal measurement has been greatly enhanced through the modification of the electrode surface with different materials, the preparation procedures of these chemically modified BFEs are rather diverse and complicated, which substantially limits the possibility of mass production since it is difficult to fabricate the same electrodes by other researchers. Besides, the supporting electrodes of conventional BFEs are relatively bulky, making them inconvenient to be deployed for on-site testing. Therefore, recent trend pertaining to the development of electrochemical sensors/electrodes for heavy metal quantification has shifted towards the direction of designing miniaturized, portable and disposable devices. Among all the attempts, Bi-coated screen-printed electrodes (Bi-SPEs) have gained a lot of attention on account of several advantages of the screen printing technology, such as the integration of three electrodes (i.e. working, reference and counter electrodes) on one substrate, the cost-effective manufacture procedure, the ease of fabricating large quantities of reproducible devices. The use of the Bi-SPEs as working electrode along with commercial reference and counter electrodes is widely reported. The Bi film formed on the surface of the SPEs can be prepared by various approaches, such as ex-situ electrodeposition in a separate Bi-containing solution prior to the measurement [66-68], in-situ electroplating Bi ions together with target metal ions [68-70], bulk modification via reducing the Bi precursor which is usually mixed with the carbon ink during printing [70-72], and surface modification through electrochemical reduction to generate Bi nanoparticles [73, 74].

To maximize the competence of the Bi-SPEs for on-site heavy metal detection, some researchers have explored the feasibility of directly employing the Bi-SPEs with screen-printed reference and counter electrodes. For example, Rico et al. [75] reported a Bi-SPE

modified by the chemically synthesized Bi nanoparticles with a printed Ag reference electrode and a carbon counter electrode for Pb, Cd, and Zn detection. In order to stabilize the Ag reference electrode, KCl was added to all the testing solutions. Under a deposition time of 120 s with 500 rpm stirring, reasonable LODs of 1.3, 1.7, 4.9 $\mu\text{g/L}$ for Pb, Cd and Zn were obtained. They also performed the testing with the aid of a commercial flow cell, from which a better LODs of 0.9, 1.3, 2.2 $\mu\text{g/L}$ for Pb, Cd and Zn were acquired. Quintana et al. [76] carried out a study to compare the analytical performance of three types of Bi-SPEs modified by different methods, namely “ex-situ electrodeposition”, “in-situ electroplating”, and “bulk modification” with bismuth oxide (Bi_2O_3), along with a printed Ag reference electrode and a graphite counter electrode to determine Pb metal ions. Instead of KCl, NaCl was used to maintain the performance of the reference electrode. The LODs of 1.0, 0.3 and 20 $\mu\text{g/L}$ were obtained for the “ex-situ”, “in-situ”, and “bulk” methods by conducting the Pb quantification with differential pulse anodic stripping voltammetry (DPASV) under a deposition time of 300, 300 and 120 s with stirring. Based on the experimental results, they found that the Bi-SPE modified by the “in-situ” method displayed the best analytical characteristics in terms of the highest sensitivity and the lowest LOD, compared with the “ex-situ” and “bulk” methods. The authors ascribed such behavior to the fact that more Pb ions could be involved in the alloy formation for the “in-situ” method as Pb and Bi ions were simultaneously deposited on the supporting electrode. By changing the electrochemical technique from the DPASV to the widely-used SWASV, they obtained LOD of 0.15 $\mu\text{g/L}$ for Pb under a deposition time of 300 s with stirring. Recently, Niu et al. [77] proposed a Bi-SPE prepared by printing the ink of Bi nanoparticle porous carbon nanocomposite for Pb and Cd detection together with a printed Ag reference electrode and a graphite counter electrode. They declared that the electroactive area of the working electrode was enlarged due to the matrix structure of the nanocomposite.

Under a deposition time of 300 s with 700 rpm stirring, the LODs of 2.3 and 1.5 $\mu\text{g/L}$ for Pb and Cd were obtained.

To achieve real on-site heavy metal measurement, the elimination of solution stirring in the course of cleaning (the removal of residual metal left on the working electrode) and deposition (the accumulation of target ions to the working electrode) steps is desirable, as it is not practical to precisely control the stirring of the testing solution during the field measurements. In 2008, Kadara et al. [78] reported a disposable Bi-SPE fabricated by direct printing the mixture of Bi_2O_3 and graphite-carbon ink onto the substrate for Pb and Cd detection along with a printed Ag/AgCl reference electrode and a carbon counter electrode. By separately calibrating the sensor in Pb and Cd solutions with the constant current stripping chronopotentiometry (CCSCP), they obtained LODs of 8 and 16 $\mu\text{g/L}$ for Pb and Cd under a deposition time of 120 s. They found that when the CCSCP was employed for simultaneous quantification of Pb and Cd, the stripping peak of Pb was unstable if the deposition potential was set more negative than -1.0 V. One year later, with the same sensor, Kadara et al. [79] achieved the simultaneous detection of Pb and Cd by simply switching the electrochemical technique to SWASV. The LODs of Pb and Cd were also improved to 5 and 2.5 $\mu\text{g/L}$ under a deposition time of 120 s. In 2010, Nie et al. [80] developed a microfluidic paper-based Bi-SPE for Pb detection, in which a carbon working electrode subsequently modified by in-situ electroplating of Bi ions, an Ag/AgCl reference electrode, and a carbon counter electrode were screen-printed on a piece of paper (polyester film), as shown in Figure 2.6. Another paper-based microfluidic channel fabricated by photolithographic patterning of the SU-8 2010 photoresist was adhered to the polyester film and a pad of cellulose blotting paper was placed near the outlet of the microfluidic channel to wick the testing solution. They compared the Pb stripping responses for the device with and without the blotting paper and found that

the device with the blotting paper exhibited the highest stripping signal. The authors ascribed such phenomenon to the enhanced accumulation efficiency of Pb ions to the working electrode on account of the convection of the analyte solution contributed by the wicking effect of the cellulose blotting paper. Under a deposition time of 120 s, the LOD of 1.0 $\mu\text{g/L}$ for Pb was attained.

Figure 2.6: Schematic illustration of the microfluidic paper-based Bi-SPE [80].

In 2010, Tan et al. [81] introduced a point-of-care Bi-SPE along with a paper disk, which contained reagents including Zn internal standard solution, Bi plating solution, and acetate buffer solution for Pb testing. The device has a screen-printed carbon working electrode modified by in-situ Bi electroplating, a printed Ag/AgCl reference electrode, and a carbon counter electrode. Preparation of the device is shown in Figure 2.7, in which the reagents were initially dropped on the paper disk and then the paper was attached to the exposed electrodes of the SPE after drying. Subsequently, a certain volume of the sample solution was released to the paper followed by the voltammetric measurement. As elaborated by the authors, the incorporation of the paper disk to the Bi-SPE brought various merits, such as the ability to quickly deliver analytes to the working electrode surface due to the porous structure of the paper, good contact between the paper and the electrodes due to good wettability of the paper, and prevention of possible particle contamination in the field measurement due to the

filtration property of the paper. Under a deposition time of 120 s, the device was able to measure Pb metal ions down to 2 $\mu\text{g/L}$ (LOD). Another innovative design of SPE was proposed by de Souza et al. [82] in 2015. In contrast to the conventional single-side SPE, they designed a new type of SPE for Pb detection, in which the three electrodes (i.e. working, reference and counter electrodes) were printed on both sides of the same substrate, as shown in Figure 2.8. Besides, the geometry of the working electrode has a thin rectangular shape (100 μm width and 20 mm length), which is also different from the conventional SPE, which usually has a circular shape. The sensor has a printed carbon-graphite working electrode, an Ag/AgCl reference electrode, and a carbon-graphite counter electrode. Having such configuration, the sensing area of the working electrode is enlarged twice and the mass transport of the metal ions to the electrode surface is also enhanced owing to the additional radial diffusion caused by the microband geometry of the working electrode in addition to planar diffusion. Under a short deposition time of 30 s, the LOD of 1.01 $\mu\text{g/L}$ for Pb was obtained.

Figure 2.7: Schematic illustration of the Bi-SPE with a paper disk impregnated with reagents [81].

Figure 2.8: Schematic illustration of the back-to-back SPE [82].

2.4.5 MEMS-fabricated electrodes

Apart from the screen printing technology, fabricating compact, portable, either disposable or reusable electrochemical devices by means of the MEMS technology, which are often termed as micro total analysis system (μ TAS) or lab-on-a-chip (LOC) devices, is becoming more and more attractive as MEMS technology reveals comparable or even superior advantages over the screen printing technology. For instance, mass production of reproducible devices can be easily accomplished because of the standardized fabrication procedure and well-established facilities. Furthermore, the price of a single sensor can be made very cheap since manufacturing of thousands of sensors with high repeatability on one piece of wafer is unchallenging. In addition, the fabrication resolution of MEMS technology is able to reach nanometer range, which is better than the screen printing technology. Recent literature pertaining to Pb, Cd and Zn detection using MEMS electrochemical sensors are reviewed as follows.

In 2008, Zou et al. [83] reported a disposable LOC heavy metal sensor for Pb detection as well as online monitoring of Cd in the soil pore and ground water. As illustrated in Figures

2.9(a) and 2.9(b), the sensor consists of two sets of three planar electrodes, i.e a Bi working electrode, an Ag/AgCl reference electrode made by electroplating with Ag and KCl solution, and an Au counter electrode, fabricated on a cyclic olefin copolymer (COC) substrate and a polymer microchannel with a rectangular chamber. The potential window of the sensor evaluated by performing cyclic voltammetry (CV) in both acetate buffer and phosphate buffered saline (PBS) buffer solutions was between -1.5 and -0.3 V with respect to the planner Ag/AgCl reference electrode, which could allow the detection of Pb, Cd, and Zn metal ions. Under a deposition time of 60 s by injecting the Pb solution with the acetate buffer into the chamber, the LOD of the sensor was 8 $\mu\text{g/L}$. As for Cd determined in the same configuration with the PBS buffer, the LOD of the sensor was 9.3 $\mu\text{g/L}$ under a deposition time of 90 s. In 2011, Jung et al. [84] presented a reusable polymer LOC sensor for continuous Pb monitoring, as shown in Figure 2.10(a), which contains a COC substrate with lithographically patterned electrodes and a microfluidic channel. In contrast to having three electrodes, they designed the sensor with only two electrodes, namely one Ag working electrode and another Ag integrated counter electrode with quasi-reference electrode (CE/QRE), as shown in Figure 2.10(b). As suggested by the authors, the Ag working electrode could be a potential sensing material for reusable heavy metal stripping in view of the monolayer formation of target metal on the Ag surface triggered by the underpotential deposition. The author asserted that the sensor was able to perform 43 consecutive SWASV runs (around 8 hours (hrs)) with less than 3% relative standard deviation (RSD) when the Pb concentrations were higher than 20 $\mu\text{g/L}$ during the calibration. They attributed such favorable behavior to the surface renewal of the working electrode caused by the re-deposition of Ag ions coming from the CE/QRE during the deposition step. However, they also found that the sensor with 100 nm Ag CE/QRE couldn't survive even 5 min. Under a deposition time of 300 s without solution flowing, the LOD of the sensor was 0.55 $\mu\text{g/L}$.

Figure 2.9: (a) Photograph of the disposable LOC heavy metal sensor [83]. (b) Photograph with an enlarged view to show the planar electrode configuration [83], where RE, reference electrode; CE, counter electrode; WE, working electrode.

Figure 2.10: (a) Photograph of the reusable polymer LOC sensor [84]. (b) Schematic illustration of the planar electrode configuration [84], where WE, working electrode; CE/QRE, integrated counter with quasi-reference electrode.

In 2012, Kokkinos et al. [85] introduced a disposable LOC sensor with integrated microelectrode array for Pb and Cd detection using the ASV approach. The sensor, as shown in Figure 2.11(a) and 2.11(b), has a Bi working microelectrode array, an Ag reference electrode which was chloridized with KCl solution prior to each measurement, and a Pt counter electrode. Regarding the performance of the microelectrode array, researchers have concluded that the ratio of the center-to-center separation of neighboring microelectrodes (d) to the radius of individual microelectrode (R_b) is the most critical parameter, which will significantly affect the diffusion regimes of the electroactive species [86-88]. They characterized different combinations of the microelectrode array by varying the values of d

and R_b using CV experiments and observed that the array with a ratio (d/R_b) of 40 showed sigmoidal voltammogram, indicating that the radial diffusion instead of linear diffusion was experienced by all microelectrodes without overlapping diffusion regimes, which provided enhanced rate of mass-transfer. Such enhancement of the accumulation of target metal ions could eliminate the solution stirring during the deposition step. Under a deposition time of 120 s, the LODs of the sensor were 0.6 and 0.7 $\mu\text{g/L}$ for Pb and Cd, respectively. In 2013, Jothimuthu et al. [89] reported a microfabricated electrochemical sensor for Zn detection. As depicted in Figure 2.12(a) and 2.12(b), the sensor comprises a glass substrate, on which an in-situ plated Bi working electrode, an Ag/AgCl auxiliary electrode (reference electrode) formed by the chlorination of a pre-electroplated Ag layer, and an Au counter electrode were patterned. A polydimethylsiloxane (PDMS) well fabricated by the standard photolithography method was attached to the glass substrate. In contrast to conducting the accumulation of Zn ions under the quiescent condition, they adopted a commercial vibrator to stir the solution during the deposition step. Under a deposition time of 600 s, they obtained the LOD of 382.3 $\mu\text{g/L}$ for Zn. The high LOD of the sensor could be due to the limited sensing area of the working electrode.

Figure 2.11: (a) Schematic illustration of the disposable LOC sensor [85], where WE, working electrode; CE, counter electrode; RE, reference electrode. (b) Optical image of one combination of the microelectrode array [85].

Figure 2.12: (a) Photograph of the microfabricated electrochemical sensor [89]. (b) A close-up of the planar electrode configuration [89], where AE, auxiliary electrode; WE, working electrode; RE, reference electrode.

In 2014, Kokkinos et al. [90] reported a compact microfabricated sensor for simultaneous Pb and Tl determination in strong acidic media. As shown in Figure 2.13(a) and 2.13(b), the sensor has a silicon dioxide (SiO_2) insulating substrate, on which a titanium (Ti) layer was initially sputtered to enhance the adhesion. Three electrodes, i.e. a Sb working electrode, an Ag reference electrode which was subsequently chloridized, and a Pt counter electrode, were fabricated by sequentially sputtering different metals with a mask. Based on the SWASV experiments, the authors found that Pb and Tl peaks were well separated when the Sb was used as the working electrode. However, an overlapping of the two peaks was observed for the sensor with Bi working electrode. The LODs of the sensor for Pb and Tl were 0.7 and 0.9 $\mu\text{g/L}$, respectively under a deposition time of 240 s with stirring. In 2015, Zhang et al. [91] presented a microfabricated LOC sensor integrated with a vibration motor for Pb and Cd detection. The sensor, as shown in Figure 2.14, consists of a glass substrate on which two Au parallel-connected working electrodes, an Ag/AgCl reference electrode, and an Au counter electrode were patterned, and a PDMS cover on which a hollow area and a circular slot were built to store the testing solution and to place the vibration motor. The working electrodes were coated with Bi film prior to the stripping analysis. As for the reference electrode, in contrast to the aforementioned literature where an Ag seed layer is firstly deposited followed

by the chlorination, they directly painted the Ag/AgCl paste on the respective position. Evaluation with CV experiments indicated reliable performance of the solid-film Ag/AgCl reference electrode in comparison to the commercial one. The authors observed a significant increase of the magnitude of stripping peaks when the motor was enabled during the deposition step. Under a deposition time of 325 s without vibration, they obtained the LODs of 1.2 and 0.7 $\mu\text{g/L}$ for Pb and Cd. With the aid of vibration, the LODs of the sensor for Pb and Cd were improved to 0.25 and 0.11 $\mu\text{g/L}$ under a deposition time of 120 s.

Figure 2.13: (a) Photograph of the compact microfabricated sensor [90]. (b) Schematic drawing to show the composition layers of the sensor [90].

Figure 2.14: Photograph of the microfabricated LOC sensor with a vibration motor [91].

To summarize, the need of performing simple, fast and accurate on-site/in-situ heavy metal measurements has driven the academic research to the direction of developing compact,

portable, disposable or reusable, and sensitive electrochemical sensors with integrated electrode configuration, rather than employing the conventional bulk electrodes. A great number of researchers have devoted tremendous efforts to investigate the analytical performances of different sensor designs with a variety of electrode materials, geometries, as well as arrangements. In general, both SPE and MEMS fabrication techniques offer promising opportunities to manufacture sensors which can partially meet the target, among which the MEMS technique is able to provide relatively more flexibility in terms of device integration. In view of the LOD (one of the key performance indicators of electrochemical sensors), a comparison pertaining to the working electrode material, detection method, deposition mode, and deposition time of different sensors with integrated solid-state electrodes fabricated by either SPE or MEMS approach is listed in Table 2.1. In regard to the working electrode material, Bi is the most popular one due to its environmentally friendly nature and the favorable stripping voltammetric characteristics. The Bi sensing film can be formed by “drop-casting of nanoparticles”, “ex-situ electrodeposition/evaporation/sputtering”, “in-situ electroplating”, and “bulk modification”, among which the “in-situ electroplating” approach exhibits lower LOD than others if the deposition is carried out under same conditions. Regarding the detection method, the SWASV method is more often selected than the DPASV one. While comparable LODs can be obtained by using both methods after optimization. As for the deposition mode, solution stirring is capable of generating much lower LOD compared with the quiescent manner. However, the involvement of solution stirring is not preferable and practical for on-site/in-situ monitoring owing to the difficulty of synchronization as well as the complexity of the setup. This tradeoff could be possibly solved by the incorporation of a vibrational motor. Unfortunately, the high vibration frequency may cause the electrodes to be quickly peeled off, shortening the lifetime of the sensor. Furthermore, such active acoustic wave will continuously propagate to the surrounding

accessories, which may cause unpredictable damage. The deposition time varies between 30 to 300 s along with different deposition modes and it seems plausible that 120 s is the minimum period of time required in order to achieve the detection of Pb less than 1 $\mu\text{g/L}$. Therefore, the challenge of designing a miniaturized sensor which is capable of achieving fast on-site/in-situ trace metal detection (which means the LOD of the sensor should be less than 1 $\mu\text{g/L}$) while excluding the use of solution stirring, still remains for academic research.

Table 2.1: Comparison of different electrochemical sensors with integrated solid-state electrodes for the determination of Pb ions

Fabrication technique	Working electrode	Detection Method	Deposition mode	Deposition time (s)	LOD ($\mu\text{g/L}$)	Reference
SPE	Synthesized Bi nanoparticles	SWASV	Stirring (500 rpm)	120	1.3	Rico et al. [75]
SPE	Synthesized Bi nanoparticles	SWASV	Flowing (38.3 $\mu\text{L/s}$)	120	0.9	Rico et al. [75]
SPE	Ex-situ plated Bi film	DPASV	Stirring ($\text{NA}^\#$)	300	1.0	Quintana et al. [76]
SPE	In-situ plated Bi film	DPASV	Stirring ($\text{NA}^\#$)	300	0.3	Quintana et al. [76]
SPE	Bulk modified with Bi_2O_3	DPASV	Stirring ($\text{NA}^\#$)	120	20	Quintana et al. [76]
SPE	In-situ plated Bi film	SWASV	Stirring ($\text{NA}^\#$)	300	0.15	Quintana et al. [76]
SPE	Bi nanoparticles with porous carbon	SWASV	Stirring (700 rpm)	300	2.3	Niu et al. [77]
SPE	Electrochemically reduced Bi_2O_3	SWASV	Quiescent	120	5.0	Kadara et al. [79]
SPE	In-situ plated Bi film	SWASV	Wicking	120	1.0	Nie et al. [80]
SPE	In-situ plated Bi film	SWASV	Quiescent	120	2.0	Tan et al. [81]
Double-side SPE	Graphite-carbon	SWASV	Quiescent	30	1.01	de Souza et al. [82]
MEMS	Evaporated Bi film	SWASV	Quiescent	60	8.0	Zou et al. [83]

MEMS	Evaporated Ag film	SWASV	Quiescent	300	0.55	Jung et al. [84]
MEMS	Sputtered Bi microelectrode array	SWASV	Quiescent	120	0.6	Kokkinos et al. [85]
MEMS	Sputtered Sb film	SWASV	Stirring (NA [#])	240	0.7	Kokkinos et al. [85]
MEMS	Ex-situ plated Bi film	DPASV	Quiescent	325	1.2	Zhang et al. [91]
MEMS	Ex-situ plated Bi film	DPASV	Vibration (~7 kHz)	120	0.25	Zhang et al. [91]

[#]NA stands for ‘not available’.

References

- [1] Sin, Y. M., Wong, M. K., & Chou, L. M. et al. (1991). A study of the heavy metal concentrations of the Singapore River. *Environmental Monitoring and Assessment*, 19(1-3), 481-494.
- [2] Goh, B. P. L., & Chou, L. M. (1997). Heavy metal levels in marine sediments of Singapore. *Environmental Monitoring and Assessment*, 44(1-3), 67-80.
- [3] Bayen, S., Thomas, G. O., & Lee, H. K. et al. (2004). Organochlorine pesticides and heavy metals in green mussel, *Perna viridis* in Singapore. *Water, Air, and Soil Pollution*, 155(1-4), 103-116.
- [4] Yuen, J. Q., Olin, P. H., & Lim, H. S. et al. (2012). Accumulation of potentially toxic elements in road deposited sediments in residential and light industrial neighborhoods of Singapore. *Journal of Environmental Management*, 101, 151-163.
- [5] World Health Organization. (2011). *Guidelines for drinking-water quality* (4th ed.). Geneva, Switzerland: World Health Organization.
- [6] Khan, D. A., Qayyum, S., & Saleem, S. et al. (2010). Lead exposure and its adverse health effects among occupational worker's children. *Toxicology and Industrial Health*, 26(8), 497-504.
- [7] Wang, Q., Zhao, H. H., & Chen, J. W. et al. (2009). Adverse health effects of lead exposure on children and exploration to internal lead indicator. *Science of the Total Environment*, 407(23), 5986-5992.
- [8] Vassileva, E., Wysocka, I., & Betti, M. (2014). Reference measurements for cadmium, copper, mercury, lead, zinc and methyl mercury mass fractions in scallop sample by isotope dilution inductively coupled plasma mass spectrometry. *Microchemical Journal*, 116, 197-205.

- [9] Martin-Camean, A., Jos, A., & Calleja, A. et al. (2014). Development and validation of an inductively coupled plasma mass spectrometry (ICP-MS) method for the determination of cobalt, chromium, copper and nickel in oral mucosa cells. *Microchemical Journal*, 114, 73-79.
- [10] Takano, S., Tanimizu, M., & Hirata, T. et al. (2013). Determination of isotopic composition of dissolved copper in seawater by multi-collector inductively coupled plasma mass spectrometry after pre-concentration using an ethylenediaminetriacetic acid chelating resin. *Analytica Chimica Acta*, 784, 33-41.
- [11] Chen, Y., Huang, L., & Wu, W. et al. (2014). Speciation analysis of lead in marine animals by using capillary electrophoresis couple online with inductively coupled plasma mass spectrometry. *Electrophoresis*, 35(9), 1346-1352.
- [12] Djedjibegovic, J., Larssen, T., & Skrbo, A. et al. (2012). Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). *Food Chemistry*, 131(2), 469-476.
- [13] Vassileva, E., & Hoenig, M. (2011). Determination of the total and extractable mass fractions of cadmium and lead in mineral feed by using isotope dilution inductively coupled plasma mass spectrometry. *Analytica Chimica Acta*, 701(1), 37-44.
- [14] Fernandez-Menendez, S., Fernandez-Sanchez, M. L., & Fernandez-Colomer, B. et al. (2016). Total zinc quantification by inductively coupled plasma-mass spectrometry and its speciation by size exclusion chromatography-inductively coupled plasma-mass spectrometry in human milk and commercial formulas: importance in infant nutrition. *Journal of Chromatography A*, 1428, 246-254.
- [15] Haglock-Adler, C. J., & Strathmann, F. G. (2015). Simplified sample preparation in the simultaneous measurement of whole blood antimony, bismuth, manganese, and zinc by inductively coupled plasma mass spectrometry. *Clinical Biochemistry*, 48(3), 135-139.

- [16] Fong, B. M. W., Siu, T. S., & Lee, J. S. K. et al. (2009). Multi-elements (aluminium, copper, magnesium, manganese, selenium and zinc) determination in serum by dynamic reaction cell-inductively coupled plasma-mass spectrometry. *Clinical Chemistry and Laboratory Medicine*, 47(1), 75-78.
- [17] ICP-MS block diagram. (n.d.). [Diagram]. Retrieved October 3, 2016, from <http://eecelabs.seas.wustl.edu/ICP-MS.aspx>.
- [18] Zhang, H. Q., Boussaad, S., & Tao, N. J. (2003). High-performance differential surface plasmon resonance sensor using quadrant cell photodetector. *Review of Scientific Instruments*, 74(1), 150-153.
- [19] Forzani, E. S., Zhang, H. Q., & Chen, W. et al. (2005). Detection of heavy metal ions in drinking water using a high-resolution differential surface plasmon resonance sensor. *Environmental Science & Technology*, 39(5), 1257-1262.
- [20] Liu, Y., Zhao, Y., & Wang, Y. et al. (2015). Polyamine-capped gold nanorod as a localized surface plasmon resonance probe for rapid and sensitive copper(II) ion detection. *Journal of Colloid and Interface Science*, 439, 7-11.
- [21] Bhattacharya, P., Chen, P., & Spano, M. N. et al. (2011). Copper detection utilizing dendrimer and gold nanowire-induced surface plasmon resonance. *Journal of Applied Physics*, 109(1), 1-6.
- [22] Sadrolhosseini, A. R., Noor, A. S. M., & Bahrami, A. et al. (2014). Application of polypyrrole multi-walled carbon nanotube composite layer for detection of mercury, lead and iron ions using surface plasmon resonance technique. *Plos One*, 9(4), 1-10.
- [23] Fen, Y. W., & Yunus, W. M. M. (2013). Utilization of chitosan-based sensor thin films for the detection of lead ion by surface plasmon resonance optical sensor. *IEEE Sensors Journal*, 13(5), 1413-1418.

- [24] Fen, Y. W., Yunus, W. M. M., & Talib, Z. A. et al. (2015). Development of surface plasmon resonance sensor for determining zinc ion using novel active nanolayers as probe. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 134, 48-52.
- [25] Bamsey, M., Graham, T., & Thompson, C. et al. (2012). Ion-specific nutrient management in closed systems: the necessity for ion-selective sensors in terrestrial and space-based agriculture and water management systems. *Sensors*, 12(10), 13349-13392.
- [26] Baig, U., & Khan, A. A. (2015). Polyurethane-based cation exchange composite membranes: preparation, characterization and its application in development of ion-selective electrode for detection of copper(II). *Journal of Industrial and Engineering Chemistry*, 29, 392-399.
- [27] Tutulea-Anastasiu, M. D., Wilson, D., & del Valle, M. et al. (2013). A solid-contact ion selective electrode for copper(II) using a succinimide derivative as ionophore. *Sensors*, 13(4), 4367-4377.
- [28] Ansari, R., Delavar, A. F., & Aliakbar, A. et al. (2012). Solid-state Cu (II) ion-selective electrode based on polyaniline-conducting polymer film doped with copper carmoisine dye complex. *Journal of Solid State Electrochemistry*, 16(3), 869-875.
- [29] Rachou, J., Gagnon, C., & Sauve, S. (2007). Use of an ion-selective electrode for free copper measurements in low salinity and low ionic strength matrices. *Environmental Chemistry*, 4(2), 90-97.
- [30] Jasinski, A., Guzinski, M., & Lisak, G. et al. (2015). Solid-contact lead(II) ion-selective electrodes for potentiometric determination of lead(II) in presence of high concentrations of Na(I), Cu(II), Cd(II), Zn(II), Ca(II) and Mg(II). *Sensors and Actuators B: Chemical*, 218, 25-30.
- [31] Huang, M. R., Rao, X. W., & Li, X. G. et al. (2011). Lead ion-selective electrodes based on polyphenylenediamine as unique solid ionophores. *Talanta*, 85(3), 1575-1584.

- [32] Lisak, G., Grygoliowicz-Pawlak, E., & Mazurkiewicz, M. et al. (2009). New polyacrylate-based lead(II) ion-selective electrodes. *Microchimica Acta*, 164(3-4), 293-297.
- [33] Yaftian, M. R., Rayati, S., & Emadi, D. et al. (2006). A coated wire-type lead(II) ion-selective electrode based on a phosphorylated calix[4]arene derivative. *Analytical Sciences*, 22(8), 1075-1078.
- [34] Hosseini, M., Abkenar, S. D., & Ganjali, M. R. et al. (2011). Determination of zinc(II) ions in waste water samples by a novel zinc sensor based on a new synthesized Schiff's base. *Materials Science and Engineering C*, 31(2), 428-433.
- [35] Singh, A. K., Mehtab, S., & Singh, U. P. et al. (2007). Tripodal chelating ligand-based sensor for selective determination of Zn(II) in biological and environmental samples. *Analytical and Bioanalytical Chemistry*, 388(8), 1867-1876.
- [36] Gupta, V. K., Chauhan, D. K., & Saini, V. K. et al. (2003). A porphyrin based potentiometric sensor for Zn^{2+} determination. *Sensors*, 3(7), 223-235.
- [37] Dumkiewicz, R., Wardak, C., & Zareba, S. (2000). Properties of the ion-selective electrode with a chelating pseudo-liquid membrane phase for zinc determination. *Analyst*, 125(3), 527-530.
- [38] Typical electrochemical cell for voltammetry. (n.d.). [Diagram]. Retrieved October 9, 2016, from <http://chem.libretexts.org/@api/deki/files/12484/=Figure11.37.jpg?revision=1>.
- [39] Linear potential sweep anodic stripping voltammetry. (n.d.). [Diagram]. Retrieved October 9, 2016, from https://upload.wikimedia.org/wikipedia/commons/9/90/Linear_Potential_Sweep_Anodic_Stripping_Voltammetry.JPG.
- [40] Kotoucek, M., Vasicova, J., & Ruzicka, J. (1993). Determination of arsenic by cathodic stripping voltammetry at a hanging mercury drop electrode. *Microchimica Acta*, 111(1-3), 55-62.

- [41] Banica, F. G., Spataru, N., & Spataru, T. (1997). Catalytic hydrogen evolution in the presence of sulfide and cobalt ions. A study by cathodic stripping voltammetry on the hanging mercury drop electrode. *Electroanalysis*, 9(17), 1341-1347.
- [42] Fernandez-Bobes, C., Fernandez-Abedul, M. T., & Costa-Garcia, A. (1998). Anodic stripping of heavy metals using a hanging mercury drop electrode in a flow system. *Electroanalysis*, 10(10), 701-706.
- [43] Decristoforo, C., Obendorf, D., & Reichart, E. et al. (1998). Determination of Sn(II) in technetium cold kits by voltammetry at the hanging mercury drop electrode (HMDE) and relevant radiopharmaceutical applications. *Nuclear Medicine and Biology*, 25(7), 675-683.
- [44] Wojciechowski, M., & Balcerzak, J. (1990). Square-wave anodic stripping voltammetry at glassy-carbon-based thin mercury film electrodes in solutions containing dissolved oxygen. *Analytical Chemistry*, 62(13), 1325-1331.
- [45] Adeloju, S. B. O., & Pablo, F. (1992). Determination of ultra-trace concentrations of tin by adsorptive cathodic stripping voltammetry on a glassy carbon mercury film electrode. *Analytica Chimica Acta*, 270(1), 143-152.
- [46] Gil, E. P., Carra, R. M. G. M., & Misiego, A. S. (1995). Determination of copper in human plasma by stripping potentiometry on a mercury film electrode in ethylenediamine medium. *Analytica Chimica Acta*, 315(1-2), 69-76.
- [47] Fischer, E., & van den Berg, C. M. G. (1999). Anodic stripping voltammetry of lead and cadmium using a mercury film electrode and thiocyanate. *Analytica Chimica Acta*, 385(1-3), 273-280.
- [48] Wang, J., Lu, J. M., & Hocevar, S. B. et al. (2000). Bismuth-coated carbon electrodes for anodic stripping voltammetry. *Analytical Chemistry*, 72(14), 3218-3222.
- [49] Hocevar, S. B., Wang, J., & Deo, R. P. et al. (2002). Potentiometric stripping analysis at bismuth-film electrode. *Electroanalysis*, 14(2), 112-115.

- [50] Kefala, G., Economou, A., & Voulgaropoulos, A. et al. (2003). A study of bismuth-film electrodes for the detection of trace metals by anodic stripping voltammetry and their application to the determination of Pb and Zn in tapwater and human hair. *Talanta*, 61(5), 603-610.
- [51] de-Carvalho, L. M., do-Nascimento, P. C., & Koschinsky, A. et al. (2007). Simultaneous determination of cadmium, lead, copper, and thallium in highly saline samples by anodic stripping voltammetry (ASV) using mercury-film and bismuth-film electrodes. *Electroanalysis*, 19(16), 1719-1726.
- [52] Baldrianova, L., Svancara, I., & Vlcek, M. et al. (2006). Effect of Bi(III) concentration on the stripping voltammetric response of in situ bismuth-coated carbon paste and gold electrodes. *Electrochimica Acta*, 52(2), 481-490.
- [53] Baldrianova, L., Agrafiotou, P., & Svancara, I. et al. (2011). The effect of acetate concentration, solution pH and conductivity on the anodic stripping voltammetry of lead and cadmium ions at in situ bismuth-plated carbon microelectrodes. *Journal of Electroanalytical Chemistry*, 660(1), 31-36.
- [54] Kruusma, J., Banks, C. E., & Compton, R. G. (2004). Mercury-free sono-electroanalytical detection of lead in human blood by use of bismuth-film-modified boron-doped diamond electrodes. *Analytical and Bioanalytical Chemistry*, 379(4), 700-706.
- [55] Cesarino, I., Gouveia-Caridade, C., & Pauliukaite, R. et al. (2010). Characterization and application of bismuth-film modified graphite-polyurethane composite electrodes. *Electroanalysis*, 22(13), 1437-1445.
- [56] Wang, Z., Li, L., & Liu, E. (2013). Graphene ultrathin film electrodes modified with bismuth nanoparticles and polyaniline porous layers for detection of lead and cadmium ions in acetate buffer solutions. *Thin Solid Films*, 544, 362-367.

- [57] Kirgoz, U. A., Marin, S., & Pumera, M. et al. (2005). Stripping voltammetry with bismuth modified graphite-epoxy composite electrodes. *Electroanalysis*, 17(10), 881-886.
- [58] Kachoosangi, R. T., Banks, C. E., & Ji, X. et al. (2007). Electroanalytical determination of cadmium(II) and lead(II) using an in-situ bismuth film modified edge plane pyrolytic graphite electrode. *Analytical Sciences*, 23(3), 283-289.
- [59] Cao, L., Jia, J., & Wang, Z. (2008). Sensitive determination of Cd and Pb by differential pulse stripping voltammetry with in situ bismuth-modified zeolite doped carbon paste electrodes. *Electrochimica Acta*, 53(5), 2177-2182.
- [60] Jia, J., Cao, L., & Wang, Z. (2007). Narion/Poly(sodium 4-styrenesulfonate) mixed coating modified bismuth film electrode for the determination of trace metals by anodic stripping voltammetry. *Electroanalysis*, 19(17), 1845-1849.
- [61] Torma, F., Kadar, M., & Toth, K. et al. (2008). Nafion[®]/2,2'-bipyridyl-modified bismuth film electrode for anodic stripping voltammetry. *Analytica Chimica Acta*, 619(2), 173-182.
- [62] Jiang, L., Wang, Y., & Ding, J. et al. (2010). An ionophore-Nafion modified bismuth electrode for the analysis of cadmium(II). *Electrochemistry Communications*, 12(2), 202-205.
- [63] Zhu, L., Xu, L., & Huang, B. et al. (2014). Simultaneous determination of Cd(II) and Pb(II) using square wave anodic stripping voltammetry at a gold nanoparticle-graphene-cysteine composite modified bismuth film electrode. *Electrochimica Acta*, 115, 471-477.
- [64] Li, Z., Chen, L., & He, F. et al. (2014). Square wave anodic stripping voltammetric determination of Cd²⁺ and Pb²⁺ at bismuth-film electrode modified with electroreduced graphene oxide-supported thiolated thionine. *Talanta*, 122, 285-292.
- [65] Lee, S., Park, S. K., & Choi, E. et al. (2016). Voltammetric determination of trace heavy metals using an electrochemically deposited graphene/bismuth nanocomposite film-modified glassy carbon electrode. *Journal of Electroanalytical Chemistry*, 766, 120-127.

- [66] Wang, J., Lu, J. M., & Hocevar, S. B. et al. (2001). Bismuth-coated screen-printed electrodes for stripping voltammetric measurements of trace lead. *Electroanalysis*, 13(1), 13-16.
- [67] Zaouak, O., Authier, L., & Cugnet, C. et al. (2009). Bismuth-coated screen-printed microband electrodes for on-field labile cadmium determination. *Electroanalysis*, 21(6), 689-695.
- [68] Rico, M. A. G., Olivares-Marin, M., & Gil, E. P. (2008). A novel cell design for the improved stripping voltammetric detection of Zn(II), Cd(II), and Pb(II) on commercial screen-printed strips by bismuth codeposition in stirred solutions. *Electroanalysis*, 20(24), 2608-2613.
- [69] Wongkaew, N., Guajardo, C., & Rijiravanich, P. et al. (2011). Use of 3-D plots to avoid mutual interference in bianalyte ASV determinations: Application to cadmium and lead detection. *Talanta*, 85(2), 1225-1228.
- [70] Lezi, N., Economou, A., & Dimovasilis, P. A. et al. (2012). Disposable screen-printed sensors modified with bismuth precursor compounds for the rapid voltammetric screening of trace Pb(II) and Cd(II). *Analytica Chimica Acta*, 728, 1-8.
- [71] Hwang, G. H., Han, W. K., & Park, J. S. et al. (2008). An electrochemical sensor based on the reduction of screen-printed bismuth oxide for the determination of trace lead and cadmium. *Sensors and Actuators B: Chemical*, 135(1), 309-316.
- [72] Lezi, N., Economou, A., & Barek, J. et al. (2014). Screen-printed disposable sensors modified with bismuth precursors for rapid voltammetric determination of 3 ecotoxic nitrophenols. *Electroanalysis*, 26(4), 766-775.
- [73] Malakhova, N. A., Stojko, N. Y., & Brainina, K. Z. (2007). Novel approach to bismuth modifying procedure for voltammetric thick film carbon containing electrodes. *Electrochemistry Communications*, 9(2), 221-227.

- [74] Lee, G. J., Kim, C. K., & Lee, M. K. et al. (2010). Advanced use of nanobismuth/naion electrode for trace analyses of zinc, cadmium, and lead. *Journal of the Electrochemical Society*, 157(7), J241-J244.
- [75] Rico, M. A. G., Olivares-Marin, M., & Gil, E. P. (2009). Modification of carbon screen-printed electrodes by adsorption of chemically synthesized Bi nanoparticles for the voltammetric stripping detection of Zn(II), Cd(II) and Pb(II). *Talanta*, 80(2), 631-635.
- [76] Quintana, J. C., Arduini, F., & Arnine, A. et al. (2011). Part I: A comparative study of bismuth-modified screen-printed electrodes for lead detection. *Analytica Chimica Acta*, 707(1), 171-177.
- [77] Niu, P., Fernandez-Sanchez, C., & Gich, M. et al. (2016). Screen-printed electrodes made of a bismuth nanoparticle porous carbon nanocomposite applied to the determination of heavy metal ions. *Microchimica Acta*, 183(2), 617-623.
- [78] Kadara, R. O., & Tothill, I. E. (2008). Development of disposable bulk-modified screen-printed electrode based on bismuth oxide for stripping chronopotentiometric analysis of lead (II) and cadmium (II) in soil and water samples. *Analytica Chimica Acta*, 623(1), 76-81.
- [79] Kadara, R. O., Jenkinson, N., & Banks, C. E. (2009). Disposable bismuth oxide screen printed electrodes for the high throughput screening of heavy metals. *Electroanalysis*, 21(22), 2410-2414.
- [80] Nie, Z., Nijhuis, C. A., & Gong, J. et al. (2010). Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip*, 10(4), 477-483.
- [81] Tan, S. N., Ge, L., & Wang, W. (2010). Paper disk on screen printed electrode for one-step sensing with an internal standard. *Analytical Chemistry*, 82(21), 8844-8847.
- [82] de Souza, A. P. R., Foster, C. W., & Kolliopoulos, A. V. et al. (2015). Screen-printed back-to-back electroanalytical sensors: heavy metal ion sensing. *Analyst*, 140(12), 4130-4136.

- [83] Zou, Z., Jang, A., & MacKnight, E. et al. (2008). Environmentally friendly disposable sensors with microfabricated on-chip planar bismuth electrode for in situ heavy metal ions measurement. *Sensors and Actuators B: Chemical*, 134(1), 18-24.
- [84] Jung, W., Jang, A., & Bishop, P. L. et al. (2011). A polymer lab chip sensor with microfabricated planar silver electrode for continuous and on-site heavy metal measurement. *Sensors and Actuators B: Chemical*, 155(1), 145-153.
- [85] Kokkinos, C., Economou, A., Raptis, I. (2012). Microfabricated disposable lab-on-a-chip sensors with integrated bismuth microelectrode arrays for voltammetric determination of trace metals. *Analytica Chimica Acta*, 710, 1-8.
- [86] Sandison, M. E., Anicet, N., & Glidle, A. et al. (2002). Optimization of the geometry and porosity of microelectrode arrays for sensor design. *Analytical Chemistry*, 74(22), 5717-5725.
- [87] Davies, T. J., & Compton, R. G. (2005). The cyclic and linear sweep voltammetry of regular and random arrays of microdisc electrodes: Theory. *Journal of Electroanalytical Chemistry*, 585(1), 63-82.
- [88] Guo, J., & Lindner, E. (2009). Cyclic voltammograms at coplanar and shallow recessed microdisk electrode arrays: guidelines for design and experiment. *Analytical Chemistry*, 81(1), 130-138.
- [89] Jothimuthu, P., Wilson, R. A., & Herren, J. et al. (2013). Zinc detection in serum by anodic stripping voltammetry on microfabricated bismuth electrodes. *Electroanalysis*, 25(2), 401-407.
- [90] Kokkinos, C., Economou, A. (2014). Disposable microfabricated 3-electrode electrochemical devices with integrated antimony working electrode for stripping voltammetric determination of selected trace metals. *Sensors and Actuators B: Chemical*, 192, 572-577.

[91] Zhang, W., Zhang, H., & Williams, S. E. et al. (2015). Microfabricated three-electrode on-chip PDMS device with a vibration motor for stripping voltammetric detection of heavy metal ions. *Talanta*, 132, 321-326.

3

BIOMIMETIC SENSOR DESIGN

This chapter starts with a detailed description of shark's olfactory sensing system, which is the source of inspiration for this project. Thereafter, the structure, dimension, and sensing principle of a shark-inspired chemical sensor are presented. Some critical aspects that have been taken into consideration when the sensor is designed and some hypotheses/merits that could be acquired through the biomimetic approach are elaborated. Finally, detailed fabrication procedures and essential fabrication parameters of the biomimetic chemical sensor are reported.

3.1 Shark's olfactory sensing system

With the impetus to address the aforementioned challenge, we would like to develop a new type of electrochemical sensor by employing the biomimetic approach. After a set of explorations and discussions, we decided to draw the inspiration from the shark's olfactory sensing system, considering that sharks have gone through millions of years of evolution. In fact, the oldest intact shark fossil found by the paleontologists, a species named as *Doliodus problematicus*, can be dated back to 409 million years ago [1]. As reported by many media, sharks exhibit extraordinary olfactory/smelling capacity in the ocean and olfaction indeed plays a pivotal role for sharks to engage food searching [2, 3], navigation [3, 4] and homing [5, 6]. Meanwhile, as suggested by Schluessel et al. [7], the olfaction may also help the juvenile sharks to survive over the early development owing to the fact that many shark species mature in a very slow speed. According to the facts reported by the National Geographic, the great white sharks are able to pinpoint one drop of blood in nearly 100 liters of water and to identify the location of blood source from few hundred meters away [8]. Such astonishing odorant sensing ability is believed to be contributed by the special morphology of their olfactory sensing systems.

The olfactory organs of sharks exhibit a large degree of diversity in terms of the shape of the nostrils, the size of the opening, and the location of the nostrils. These differences are assumed to be related to their surrounding environments as well as the foraging strategies. Sharks can be roughly divided into two groups, i.e. benthic sharks and pelagic sharks. The research conducted by Schluessel et al. [9] concluded that benthic sharks like to swim slowly and search for hidden or sluggish prey. Hence their incurrent nostrils, as depicted in Figure 3.1(a), are circular and wide, which are often positioned near the mouth, allowing them to have more space to be exposed to the water. On the contrary, pelagic sharks prefer to swim at

high speed in the open ocean areas for food foraging. Therefore, they have evolved oval and narrow incurrent nostrils, which are often situated far away from the mouth, as shown Figure 3.1(b). It is hypothesized that the harmful impact of water (e.g. high pressure) to the tissues inside the olfactory organs can be greatly reduced for smaller nostrils when sharks cruise at high speed. They also measured the mass of the olfactory bulb, the mass of the olfactory rosette, the number of the olfactory lamellae, and the surface area of the olfactory epithelium to study the relationship of the olfactory morphology to both habitat occupancy and lifestyle using the statistical method of generalized least squares phylogenetic regression [10]. Although there was no significant difference between the mass of the olfactory bulb and the habitat type when the body length was used as a covariate, the mass of the olfactory rosette showed a clear relevance to the living habitat, in which pelagic sharks have heavier rosette compared to the benthic ones. As for the number of the olfactory lamellae, pelagic sharks (from 84 to 231 with an average of 60) possess significantly more sensory lamellae than the benthic ones (from 58 to 187 with an average of 40). Regarding the surface area of the olfactory epithelium, pelagic sharks have much larger sensing epithelial area relative to the benthic ones. All these statistical comparisons demonstrated that the morphology of shark's olfactory sensing systems is predominantly oriented by the pressure coming from the preferred habitat in the course of evolution. In general, pelagic sharks have developed more sensitive chemoreception capacity than the benthic sharks.

Figure 3.1: External morphology of the olfactory organs of (a) a benthic shark (brownbanded bamboo shark) and (b) a pelagic shark (nervous shark) [9], where the scale bar represents 1 cm.

The conclusion is also supported by Timm et al. [11], where they conducted a thorough comparative research to investigate how olfactory morphology of benthic and pelagic sharks is related to the odorant processing mechanism. The authors picked up both internal and external anatomical data together with computed tomography of twenty-three sharks to perform a series of statistical analyses including principal component analysis, full factorial multivariate analysis of variance, post hoc tests and independent sample t-tests. What they found was that pelagic sharks have larger narial length, mouth length, and nostril angle than benthic ones. The external nasal structures of pelagic and benthic sharks reveal different characteristics with some variations. According to their interpretation, pelagic sharks rely on the so-called pitot mechanism, in which water is directed inside to the olfactory organs due to the orthogonal relationship between the angle of the incurrent nostril and that of the excurrent nostril. The pressure difference resulted from the pitot-like arrangement accelerates the water to move into the olfactory cavity, when pelagic sharks swim towards the upstream current. In addition, the nostrils of pelagic sharks are located at the tip of the streamlined head, which will generate a thinner boundary layer, thereby improving the transport of odorant molecules to the olfactory cavity. However, the olfactory organs of benthic sharks are actually

connected to their mouth, which indicates that the water ventilation in the olfactory cavity is mainly induced by the respiration activity.

Given the above discussions, the design of a sensitive artificial chemical sensor should try to mimic the olfactory sensing systems of the pelagic sharks. For the purpose of improving the efficiency of sensor design, acquiring sufficient and detailed knowledge pertaining to the olfactory morphology of pelagic sharks is needed. Zeiske et al. [12] studied the morphology of the olfactory organs of lemon shark by using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). As shown in Figure 3.2, a pair of olfactory organs with each of them comprising one incurrent nostril (in) and one excurrent nostril (en) is present on the ventral side of the head of the lemon shark. The olfactory organs are not connected to the mouth, which distinguishes them from benthic sharks as well as air-breathing vertebrates. This fact may imply that the function of the olfactory organs of pelagic sharks is totally used for collecting odorant information rather than breathing under the water. Incurrent nostril has an oval-shaped wide entrance with its inner wall extending to the olfactory rosette (or). A triangular nasal flap (nf) partially connects the incurrent nostril to the excurrent one, which may facilitate the water movement during forward swimming. The excurrent nostril, on the other hand, forms a narrow slit-like opening compared to the incurrent nostril.

Figure 3.2: Ventral view of the head of a lemon shark and an enlarged view to show one olfactory organ [12], where in, incurrent nostril; or, olfactory rosette; nf, nasal flap; en,

excurrent nostril. The red dotted line indicates the position through which the olfactory organ is sliced to show the internal anatomy (as depicted in Figure 3.3) and the solid yellow arrow indicates the view direction.

If the olfactory organ is sliced along the red dotted line indicated in Figure 3.2, the internal anatomy of the olfactory organ can be viewed from the cross-sectional direction, as depicted in Figure 3.3. Two rows of a number of olfactory lamellae (ol) separated by a central raphe (r) aggregate with a rosette manner inside the olfactory cavity. The inner margins (im) of all olfactory lamellae gather together to form a tubular inlet chamber (ic). While the outer margins of the olfactory lamellae stick to the cavity wall (cw) and spread from the raphe towards the gallery (g), along which a gradually broad peripheral canal (pc) is created. A gap system with multiple narrow channels is formed between the neighboring lamellae. The secondary folds (sf) of the olfactory lamellae are extended inside the channels, which greatly enlarge the total area of the olfactory lamellae. When pelagic sharks actively swim forward in the ocean, water is initially pumped into the inlet chamber contributed by the pressure difference created between the incurrent nostril and the excurrent one. After that, the water is guided into the gap system at which odorant molecules dissolved in the water will be in intimate contact with the olfactory lamellae and then the water is discharged into the gallery through the peripheral canal. The flow path of the water through the olfactory organ of the silky shark, another representative pelagic shark which is highly energetic and mobile, is schematically presented in Figure 3.4. Movement of the water inside the gap system is able to promote the convection effect of the molecules transport to the sensing areas of the olfactory lamellae, which is much more efficient as compared with the diffusion effect, a spontaneous motion induced by the concentration gradient. As calculated by Cox J. P. L., around 10 min is required in order to make odorant-like molecules, such as amino acids, steroids, and prostaglandins, to diffuse 1 mm distance in water [13].

Figure 3.3: Cross-sectional SEM image of the olfactory organ and an enlarged view of half of the olfactory rosette to show the internal anatomy [12], where vf, valve flap; ic, inlet chamber; r, raphe; ol, olfactory lamella; g, gallery; sf, secondary fold; pc, peripheral canal; im, inner lamellar margin; cw, cavity wall. Areas denoted by “I” and “II” are shown in Figure 3.5.

Figure 3.4: Schematic drawing of the gap system of the silky shark to show the flow path of the water (solid arrows) through the olfactory organ [12].

As shown in Figure 3.5(a) and 3.5(b), SEM images with high magnification reveal the histology of the olfactory lamellae. It can be observed that both nonsensory epithelium (ne) and sensory epithelium (se) are distributed on the surface of the olfactory lamellae. The nonsensory epithelium is usually located near the inner lamellar margins as well as the peripheral canals, whereas the sensory epithelium covers the majority areas of the olfactory

lamellae, including the secondary folds. The nonsensory epithelium can be easily identified by the presence of numerous goblet cells which always bear high-density, short and round microvilli. As for the sensory epithelium, a pseudostratified and columnar arrangement of the cells can be noticed, where the non-sensory ciliated cells with multiple cilia and flat apices dominate the most surfaces. The major receptor cells, as illustrated by the red box in Figure 3.4(b), have a swelling/knob structure protruded from the epithelial surface, on which a tuft of microvilli are scattered. The morphological distribution of the protruding sensory receptor cells embedded on both sides of the olfactory lamellae together with the massive surface area supplied by the secondary folds significantly increases the possibilities of odorant molecules to be captured, which renders pelagic sharks to possess super keen olfactory sensing capability.

Figure 3.5: SEM image with high magnification [12] to show the enlarged area of (a) “I” and (b) “II” denoted in Figure 3.3, where se, sensory epithelium; ne, nonsensory epithelium.

3.2 Design of biomimetic chemical sensor

Inspired by the intriguing morphology of the olfactory sensing system of pelagic sharks, an innovative chemical sensor is proposed in order to achieve fast and accurate on-site/in-situ trace metal detection. The sensor consists of two parts as shown in Figure 3.6(a) and 3.6(b), where the top part is a microfluidic channel which serves as a holder for the sample solution

as well as a chamber for the electrochemical reaction. The bottom part is a sensor base to place three on-chip electrodes along with the contact pads. A micropillar electrode array is placed on top of the bottom working electrode. Assembly marks located at four corners of the sensor base are used to provide the alignment reference for combining the top part with the bottom one. The sensing principle of the biomimetic chemical sensor is based on one of the electroanalytical voltammetric methods – ASV, which is particularly described in section 2.2.4. Briefly, the sample solution containing different kinds of heavy metal ions is firstly pumped into the reaction chamber through the inlet of the microfluidic channel, during which the cleaning and deposition steps will be carried out. Subsequently, the flowing solution is stopped to perform the quiescent and stripping steps, after which a new measurement will commence again with fresh sample solution being pumped into the reaction chamber.

Figure 3.6: (a) Schematic drawing of the biomimetic chemical sensor consisting of a top microfluidic channel and a bottom sensor base. (b) An enlarged view of the sensor base to show the reference, working and counter electrodes.

To determine some reference data for designing the biomimetic chemical sensor, the dimensions of the olfactory lamellae are firstly estimated based on SEM image of the olfactory rosette of a lemon shark, as shown in Figure 3.7(a). The red arrows denote the order of the olfactory lamellae, counting from the inlet chamber towards the direction of the gallery, which is also the outlet chamber. The length of 28 pieces of the olfactory lamellae is estimated by measuring the distance between two ends of each lamella, denoted by the green arrow. Figure 3.7(b) shows the measured results, in which the length ranges from 0.36 to 1.75 mm with an average value of 1.2 mm. The width of all the olfactory lamellae is also estimated by measuring the distance between two edges of each lamella, denoted by the yellow arrow. Observed from Figure 3.7(a), the width of each lamella slightly increases along the length, which varies between 0.05 and 0.15 mm. The width of the inlet chamber of the olfactory rosette, denoted by the blue arrow, is measured about 1.05 mm. Similarly, the width of the outlet chamber of the olfactory rosette, denoted by the brown arrow, is estimated around 0.98 mm.

Figure 3.7: (a) Estimation of dimensions of the olfactory lamellae based on SEM image [12] of the olfactory rosette of a lemon shark, where g, gallery; sf, secondary fold; pc, peripheral canal; ic, inlet chamber; im, inner lamellar margin; cw, cavity wall. The red, green, yellow, blue, and brown arrows denote the order of the lamella, the length of the lamella, the width of the lamella, the width of the inlet chamber, and the width of the outlet chamber, respectively.

(b) The estimated length of 28 pieces of the olfactory lamellae corresponding to the order denoted in Figure 3.7(a).

Based on the aforementioned estimation, the dimensions of the chemical sensor are chosen as follows. The microfluidic channel, as outlined in Figure 3.8(a), has a rectangular inlet chamber with width of 1 mm and length of 4 mm. The width of the inlet chamber (1 mm) is selected as close as the dimension of the inlet chamber of the olfactory rosette (1.05 mm). The length of the inlet chamber (4 mm) is defined 4 times longer than the width to ensure that a stable flow can be formed inside the inlet chamber before entering into the reaction chamber to eliminate any uncertainty caused by the flow. Following the inlet chamber, a semicircular reaction chamber with radius of 2.4 mm is designed. This reaction chamber imitates the shape of the olfactory rosette and the radius of the reaction chamber (2.4 mm) is roughly selected as 2 times longer than the average length of the olfactory lamellae (1.2 mm). Behind the reaction chamber, a rectangular outlet chamber with width of 1 mm and length of 2 mm is connected. The width of the outlet chamber (1 mm) is comparable to the dimension of the outlet chamber of the olfactory rosette (0.98 mm). In order to minimize overall footprint of the sensor, the length of the outlet chamber (2 mm) is specified only 2 times longer than the width. The height of the microfluidic channel is selected as 0.12 mm. According to the proposed design (Figure 3.6), the height of the microfluidic channel should be same as the height of the micropillar electrode array. To make the micropillar electrodes, SU-8 2100 negative photoresist is designated to shape the core of each pillar for the reason that SU-8 is able to produce various structures with stable mechanical and thermal properties [14-17]. Therefore, the height of the microfluidic channel (0.12 mm) is determined by the maximum thickness of a uniform SU-8 layer that was obtained through preliminary experiment of spin-coating.

Figure 3.8: Dimensions of (a) the top microfluidic channel and (b) the three on-chip electrodes placed on the bottom sensor base, where only 12 micropillars are shown in order to improve the clarity.

For the sensor base, as depicted in Figure 3.8(b), it consists of three on-chip electrodes. Specifically, an inner semi-annulus with a radius of the smaller circle of 0.6 mm and the radius of the larger circle of 0.9 mm is the reference electrode. A middle semi-annulus with radius of the smaller circle of 1 mm and the radius of the larger circle of 1.5 mm is the bottom working electrode, on which an array consisting of 24 micropillar electrodes with diameter of 0.1 mm and height of 0.12 mm is evenly placed with the spacing of 0.16 mm along the semi-arc with radius of 1.25 mm. An outer semi-annulus with a radius of the smaller circle of 1.6 mm and the radius of the larger circle of 1.9 mm is the counter electrode. All dimensions of the three on-chip electrodes are determined in accordance with the boundary of the reaction chamber. The location of the micropillar electrodes is specified in the middle section of the semicircular reaction chamber. The diameter of the micropillar electrodes (0.1 mm) is selected based on the width of the olfactory lamellae (between 0.05 and 0.15 mm). The height of the micropillar electrodes (0.12 mm) is chosen as same as that of the microfluidic channel (0.12 mm), as discussed above. The spacing between adjacent

micropillar electrodes is decided in consideration of the feasibility of fabrication to avoid uncontrolled sticking of the base of SU-8 micropillars.

There are some critical aspects that have been taken into consideration when the chemical sensor is designed. Following points will elaborate these aspects as well as some possible merits that could be acquired in view of our hypotheses.

1) As shown in Figure 3.9(a), apart from the bottom flat working electrode, a number of 3D micropillars with both side and top surfaces coated by sensing materials are also incorporated as a working electrode array. This kind of design is very different from the conventional electrochemical sensors, where most of them only have 2D planar working electrodes as reviewed in the previous section 2.4. Such idea of developing 3D micropillar working electrode array is intended to mimic the morphological distribution of the olfactory receptor cells of the pelagic sharks as the cells always protrude outside from the surface of the sensory epithelium, as depicted in Figure 3.5(b). By extending single-layer planar working electrode into a stratified columnar working electrode array, the total sensing area that is subjected to sample solution can be greatly enlarged, thereby possibly enhancing the sensitivity of the biomimetic chemical sensor since the magnitude of stripping current of ASV is directly proportional to the surface area of the working electrode.

2) According to the working principle of three electrode system, the function of the reference electrode is to provide a reference point from which the potential of the working electrode can be precisely controlled and measured, which means no current will pass through the reference electrode throughout the electrochemical experiment. To put it another way, no reaction will happen on the reference electrode during the measurement. Therefore, in our

design, the reference electrode, as illustrated in Figure 3.9(a), is placed near the end of the inlet chamber, making sample solution to firstly pass through it. The working electrode is the place where the reaction of interest will take place. As a result, the working electrode of the biomimetic chemical sensor is placed just behind the reference electrode. The function of the counter electrode in the three electrode system is to balance the charge added or removed from the working electrode, which means current will pass through between the working electrode and the counter electrode. Either reduction or oxidation of solvent/supporting electrolyte present in the sample solution may undergo on the counter electrode. Hence, the counter electrode of the biomimetic chemical sensor is placed downstream with respect to the working electrode in such a way that any disturbance produced by the redox reaction happening on the counter electrode could be diminished.

3) The integration of the microfluidic channel with the micropillar working electrode array is aimed at mimicking the gap system that is formed by the facing olfactory lamellae inside the olfactory rosette. For the lemon shark, when water is sucked into the olfactory rosette under the effect of pressure difference, it will immediately spread along the interspace of the facing olfactory lamellae, as illustrated in Figure 3.9(b). One outstanding advantage of this structure is that all olfactory lamellae will contribute to the detection of odorant molecules since olfactory receptor cells that are scattered on both sides of each lamella can give full play to their competence, maximizing the molecule collection efficiency with a limited volume of water. In a similar manner, when the sample solution is pumped into the inlet chamber of the microfluidic channel, it will be directed to the subchannels formed by neighboring micropillar electrodes. Therefore, all micropillar working electrodes can participate in the accumulation process of metal ions, which could possibly minimize the total volume of the required sample solution. However, a sensor with only flat working electrode will need more

volume of sample solution as the majority of the sample solution is wasted. Furthermore, the 3D shape of the micropillar working electrodes enables sensing surface to extend beyond the stagnation region developed by the flowing solution along the cross-sectional plane of the microfluidic channel, which could possibly enhance the ion collection efficiency. On the other hand, the planar working electrode will be either partially or entirely concealed inside the stagnation region as it is usually located in the lower section of the microfluidic channel, which would result in reduced ion collection efficiency.

4) It can be noticed that the micropillar working electrode array is organized in a curved or parabolic fashion rather than a straight line. Designing such structure is to draw inspiration from the arrangement of the olfactory lamellae, as shown in Figure 3.7(b). It seems that the distribution of the olfactory lamellae inside the olfactory rosette follows a pseudo-parabolic manner. Such variation may have some sort of hydrodynamic advantage to facilitate water circulation, resulting in reinforcement of the transport of odorant molecules to the sensory epithelium. Another merit is that a curved/parabolic arrangement would accommodate more number of micropillar electrodes as compared to a straight-line one within the same footprint, if the dimensions of the micropillar working electrode array (i.e. diameter of individual pillar, height of individual pillar, space between adjacent pillars, and number of rows of pillars) are kept same.

Figure 3.9: (a) Schematic drawing of the biomimetic chemical sensor to show flow path of sample solution (solid black arrows) through the microfluidic channel, where RE, reference electrode; WE, working electrode; CE, counter electrode. (b) Schematic drawing of the gap system of silky shark to show flow path of the water (solid black arrows) through the olfactory organ [12].

3.3 Fabrication of biomimetic chemical sensor

3.3.1 Fabrication of sensor base

Fabrication of the proposed biomimetic chemical sensor is utterly carried out with the aid of MEMS technology. The sensor base after fabrication consists of multiple layers as shown in Figure 3.10, where a Si substrate (500 μm) serves as a supporting basis for the upper structure and a SiO_2 layer (1 μm) will provide proper insulation among the three electrodes, i.e. reference electrode (RE), working electrode (WE), and counter electrode (CE). A layer of Au (300 nm) is basically to supply electrical connection to each electrode, below which a layer of Cr (100 nm) is added to strengthen the adhesion force between the Au layer and the underneath SiO_2 insulation layer. A layer of Ag/AgCl (500 nm) is deposited on top of a predetermined portion of the Au layer, which plays the role of the reference electrode. Another layer of Au (600 nm) is deposited next to the reference electrode as the bottom working electrode, above which a layer of SU-8 (120 μm) is patterned to form the core of the micropillar electrodes. A thin film of Au (200 nm) is coated on both top and side surfaces of

all the SU-8 micropillars, which functions as the 3D micropillar working electrode array. No modification is made to the remaining Au layer, which is used as the counter electrode.

Figure 3.10: Schematic drawing of the cross section of the sensor base after fabrication, where RE, reference electrode; WE, working electrode; CE, counter electrode.

As shown in Figure 3.11, fabrication of the sensor base was initiated by depositing 1 μm SiO₂ insulation layer using the plasma-enhanced chemical vapor deposition (PECVD) on a brand new p-type <100> Si wafer with a diameter of 4 inches (step 1). The wafer was then thoroughly cleaned by acetone along with isopropanol and rinsed by deionized (DI) water. The washed wafer was dried in a rotational wafer spin dryer with flowing nitrogen (N₂) gas, after which the wafer was transferred into a hexamethyldisilazane (HMDS) promoter for treatment in order to enhance the adhesion of the subsequent spinning photoresist. After cooling, the wafer was placed inside the chamber of a spin-coater and around 5 mL AZ 9260 photoresist was dropped on the surface of the wafer. A fine adjustment was performed to align the center of the wafer with the center of the rotational shaft in order to have a photoresist layer with uniform thickness. Next step was to spin a 5 μm layer of the photoresist on top of the insulation layer under 5000 rpm rotation speed for 30 s, followed by baking on a hotplate at 110 °C for 4 min (step 2). The baked photoresist was allowed to cool down to the room temperature and was exposed to 365 nm i-line ultraviolet (UV) light. After

exposure, the wafer together with the exposed photoresist was totally immersed in a sufficient amount of 400K photoresist developer solution, in which strong agitation was provided to ensure the geometric pattern printed on the plastic photomask can be precisely transferred onto the photoresist. As the AZ 9260 is a positive type of light-sensitive resist, the portions that were exposed to the UV light were dissolved by the developer solution, resulting in some opened areas at certain locations (step 3). After rinsing with DI water and being dried by N₂ gas, the wafer with the patterned photoresist was placed in the chamber of a DC-magnetron sputter. A metal layer comprising 100 nm Cr and 300 nm Au was then coated on the top surface of the entire wafer (step 4). After that, the wafer together with the sputtered metal was completely dipped into acetone and kept for 12 hrs. All remaining photoresist left on the wafer and the sputtered metal attached on the photoresist were totally removed by the acetone (step 5). Subsequently, the wafer was again rinsed with DI water and dried by N₂ gas to wash away all other residues. Till here, fabrication of the base electrode layer (which is also the counter electrode layer) was accomplished. Following the same procedures, the bottom working electrode layer of 600 nm Au (step 6 to step 9) and the reference electrode layer of 200/300 nm Ag/AgCl (step 10 to step 13) and were fabricated by defining different opened areas with different plastic photomasks.

1. Deposition of 1 μm SiO_2 insulation layer

2. Spin-coating of 5 μm positive photoresist

3. Photolithography of photoresist to form first-layer pattern

4. Sputtering of 100 nm Cr together with 300 nm Au

5. Removal of photoresist to expose base electrode layer

6. Spin-coating of another 5 μm positive photoresist

7. Photolithography of photoresist to form second-layer pattern

8. Sputtering of 600 nm Au

9. Removal of photoresist to expose bottom working electrode layer

10. Spin-coating of another 5 μm positive photoresist

11. Photolithography of photoresist to form third-layer pattern

12. Sputtering of 200 nm Ag and evaporating of 300 nm AgCl

Figure 3.11: Detailed fabrication procedures of the sensor base of the biomimetic chemical sensor.

3.3.2 Fabrication of micropillar electrode array

After cleaning the wafer with acetone and isopropanol and rinsing it with DI water, the wafer was initially placed on a hotplate to perform a heat treatment at 80 °C for 10 min in order to evaporate the adsorbed water molecules. When the wafer cooled down to the room temperature, the spin-coating process was commenced inside the spin coater. A thick layer of 120 μm SU-8 negative photoresist was spun to cover the entire wafer under 3000 rpm rotation speed with 300 rpm/s acceleration for 30 s (step 14 in Figure 3.11). After that, the wafer together with the coated SU-8 photoresist was placed on a hotplate to conduct soft

baking. For the sake of maintaining the SU-8 thick layer with good uniformity in thickness, the baking temperature and the baking time were comprehensively optimized. After a series of trials, the baking condition was determined, where the temperature was firstly ramped from 50 to 65 °C for 5 min, further ascended to 80 °C for another 5 min, and finally stopped at 90 °C for 30 min. When the pre-baked SU-8 layer completely reached to the room temperature, photolithography with UV light was implemented after which post baking was started from 50 °C. The baking condition was similar to the soft baking except for last baking time, which was 15 min instead of 30 min. Once post baking was finished, the wafer together with the exposed SU-8 photoresist was completely dipped in the SU-8 developer solution. Unlike the AZ 9260 positive photoresist, SU-8 is a negative type of light-sensitive resist, which means the portions that were not exposed to the UV light were dissolved by the developer solution, forming an array of equally separated micropillars (step 15). Thereafter, the wafer was rinsed with fresh developer solution along with isopropanol and dried by N₂ gas to fully remove the residual SU-8 photoresist. Following step was to spin a new layer of 5 µm AZ 9260 positive photoresist and the similar photolithography process as described in the previous paragraph was performed to make a pattern in such a way that both and counter electrodes were enclosed by the AZ 9260 photoresist, only leaving the bottom working electrode to be uncovered (step 16). Subsequently, the wafer placed on an inclined support was put inside the chamber of the DC-magnetron sputter and a 200 nm thin film of Au was sputtered on both top and side surfaces of all the micropillars to generate the 3D micropillar working electrode array (step 17). The final step was to clear away the remaining positive photoresist along with the coated Au film on it using the acetone (step 18). By rinsing the wafer with DI water and drying it with N₂ gas, fabrication of the sensor base of the proposed biomimetic chemical sensor was accomplished. Figures 3.12(a) and 3.12(b) show the photograph of one chemical sensor before and after fabricating the micropillar working

electrode array, which corresponds to the step 13 and 18 depicted in Figure 3.11, respectively. A close-up image of the micropillar working electrode array taken by standard SEM equipment is shown in Figure 3.12(c), in which it can be clearly observed that an array of 24 equally-spaced micropillars with good uniformity was successfully fabricated with the aid of MEMS technology.

Figure 3.12: Photograph of the biomimetic chemical sensor (a) before and (b) after fabrication of the micropillar working electrode array using MEMS technology. (c) SEM image of the micropillar working electrode array.

3.3.3 Fabrication of microfluidic channel

As shown in Figure 3.13, fabrication of the microfluidic channel was initiated by spin-coating a 120 μm layer of SU-8 negative photoresist on a brand new Si wafer with a diameter of 4 inches under 3000 rpm rotation speed with 300 rpm/s acceleration for 30 s (step 1). Thereafter, soft baking of the coated SU-8 photoresist on a hotplate was carried out using the same baking protocol of the SU-8 micropillars, as mentioned in the previous paragraph. Before the photolithographic operation, the wafer together with the baked SU-8 photoresist

was stored in a closed chamber at room temperature in order to diminish the thermal stress induced during the soft-baking process until a uniform layer of SU-8 photoresist was obtained. After exposure to the UV light, the post baking was conducted following the same baking protocol of the SU-8 micropillars. The mold of the microfluidic channel was shaped by using SU-8 developer solution to wash off the unexposed photoresist in specific areas, after which the wafer was rinsed with fresh developer solution along with isopropanol and dried by N₂ gas (step 2). Thereafter, PDMS solution A and solution B were vigorously mixed in a ratio of 10:1 to turn the original liquid into the material with appropriate viscosity. The blended solution was put inside the vacuum chamber for 3 hrs to perform the degassing process as the degree of degasification could have a great impact on the generation of air bubbles in the mixed PDMS solution. Subsequently, the degassed solution was slowly poured into the SU-8 mold, which was confined in a petri dish with ventilation apertures (step 3). The petri dish was then shifted to an automatically controlled oven to proceed with constant heating at 75 °C for 3 hrs. Once the baked PDMS was peeled off from the SU-8 mold, the fabrication of the microfluidic channel was accomplished (step 4). Finally, the PDMS microfluidic channel was attached to the sensor base using adhesive glue to build the proposed biomimetic chemical sensor. Figure 3.14(a) and 3.14(b) separately depict an SU-8 mold fabricated on the Si substrate and a PDMS microfluidic channel after peeling off from the SU-8 mold. Figure 3.14(c) shows the photograph of a fabricated biomimetic chemical sensor after assembling the microfluidic channel with the sensor base. These photographs (Figure 3.12 and 3.14) distinctly demonstrate the feasibility of adopting MEMS technology to manufacture miniaturized chemical sensors with favorable repeatability and accuracy.

Figure 3.13: Detailed fabrication procedures of the microfluidic channel of the biomimetic chemical sensor.

Figure 3.14: Photograph of (a) an SU-8 mold, (b) a PDMS microfluidic channel, and (c) a fabricated biomimetic chemical sensor after assembly, where RE, reference electrode; WE, working electrode; CE, counter electrode.

References

- [1] World's oldest shark fossil found. (2003). In *National Geographic News*. Retrieved from http://news.nationalgeographic.com/news/2003/10/1001_031001_sharkfossil.html.
- [2] Theiss, S. M., Hart, N. S., & Collin, S. P. (2009). Morphological indicators of olfactory capability in wobbegong sharks (Orectolobidae, Elasmobranchii). *Brain, Behavior and Evolution*, 73(2), 91-101.
- [3] Montgomery, J. C., & Walker, M. M. (2001). Orientation and navigation in elasmobranchs: which way forward?. *Environmental Biology of Fishes*, 60(1-3), 109-116.
- [4] Nosal, A. P., Chao, Y., & Farrara, J. D. et al. (2016). Olfaction contributes to pelagic navigation in a coastal shark. *Plos One*, 11(1), 1-17.
- [5] Edren, S. M. C., & Gruber, S. H. (2005). Homing ability of young lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes*, 72(3), 267-281.
- [6] Gardiner, J. M., Whitney, N. M., & Hueter, R. E. (2015). Smells like home: the role of olfactory cues in the homing behavior of blacktip sharks, *Carcharhinus limbatus*. *Integrative and Comparative Biology*, 55(3), 495-506.
- [7] Schluessel, V., Bennett, M. B., & Bleckmann, H. et al. (2010). The role of olfaction throughout juvenile development: functional adaptations in elasmobranchs. *Journal of Morphology*, 271(4), 451-461.
- [8] Blood in the water facts. (2013). In *National Geographic Wild*. Retrieved from <http://channel.nationalgeographic.com/wild/dead-or-alive/articles/blood-in-the-water-facts/>.
- [9] Schluessel, V., Bennett, M. B., & Bleckmann, H. et al. (2008). Morphometric and ultrastructural comparison of the olfactory system in elasmobranchs: the significance of structure-function relationships based on phylogeny and ecology. *Journal of Morphology*, 269(11), 1365–1386.

- [10] de Esteban-Trivigno, S., & Kohler, M. (2011). New equations for body mass estimation in bovids: testing some procedures when constructing regression functions. *Mammalian Biology*, 76(6), 755–761.
- [11] Timm, L. L., & Fish, F. E. (2012). A comparative morphological study of head shape and olfactory cavities of sharks inhabiting benthic and coastal/pelagic environments. *Journal of Experimental Marine Biology and Ecology*, 414, 75–84.
- [12] Zeiske, E., Theisen, B., & Gruber, S. H. (1987). Functional morphology of the olfactory organ of two carcharhinid shark species. *Canadian Journal of Zoology*, 65(10), 2406-2412.
- [13] Cox, J. P. L. (2008). Hydrodynamic aspects of fish olfaction. *Journal of the Royal Society Interface*, 5(23), 575-593.
- [14] Conradie, E. H., & Moore, D. F. (2002). SU-8 thick photoresist processing as a functional material for MEMS applications. *Journal of Micromechanics and Microengineering*, 12(4), 368-374.
- [15] Kim, K., Park, D. S., & Lu, H. M. et al. (2004). A tapered hollow metallic microneedle array using backside exposure of SU-8. *Journal of Micromechanics and Microengineering*, 14(4), 597-603.
- [16] del Campo, A., & Greiner, C. (2007). SU-8: a photoresist for high-aspect-ratio and 3D submicron lithography. *Journal of Micromechanics and Microengineering*, 17(6), R81-R95.
- [17] Jacot-Descombes, L., Gullo, M. R., & Cadarso, V. J. et al. (2012). Fabrication of epoxy spherical microstructures by controlled drop-on-demand inkjet printing. *Journal of Micromechanics and Microengineering*, 22(7), 1-8.

4

CHARACTERIZATION OF BIOMIMETIC CHEMICAL SENSOR

This chapter mainly focuses on the characterization of the on-chip reference electrode as well as the bio-inspired micropillar electrode array. Two types of on-chip reference electrodes, one of which is made of a layer of sputtered Ag and the other is made of evaporated AgCl coated on a sputtered Ag layer, are investigated in the aspects of a) stability over a range of scan rates, b) stability over a prolonged period of measurement, and c) response consistency over multiple reference electrodes. As for the bio-inspired micropillar electrode array, electrical connection, surface property, electrochemical sensing ability, and total Au coverage area are separately evaluated.

4.1 Characterization of on-chip reference electrode

4.1.1 Background and theory

For an electrochemical sensor to perform the accurate and reliable measurement, having a reference electrode with consistent potential is a requisite. This predetermined potential is the reference point to which either the controlled or the measured potential of the working electrode is compared. Generally, commercial Ag/AgCl reference electrode with aqueous inner electrolyte (3 M/saturated NaCl or KCl) is the most welcome one as the preparation of the electrode is relatively simple and inexpensive. The nontoxic and robust properties of the Ag/AgCl reference electrodes also promote its popularity of usage. However, the bulky size together with the involvement of aqueous electrolyte inside the electrode hinders the ability to be directly integrated into the compact and portable electroanalytical LOC devices. To circumvent this issue, solid-state thin-film Ag/AgCl reference electrodes without aqueous electrolyte, which are often termed as quasi Ag/AgCl reference electrode or pseudo Ag/AgCl reference electrode fabricated via the SPE or the MEMS technology, have been developed, as reviewed in section 2.4. Because of the absence of proper inner electrolyte, thin-film Ag/AgCl reference electrodes don't have the thermodynamic equilibrium, which technically deviates from the true reference electrodes. Therefore, the potential may have a drift in the course of prolonged/multiple measurements under certain circumstances as some thin-film reference electrodes can be easily polarized. However, there are still valuable advantages of the quasi/pseudo thin-film reference electrodes. For example, the simplicity of the composition allows simultaneous fabrication of three electrodes on a single substrate, leading to great prosperity of the compact and disposable electrochemical sensors. In addition, the small footprint (in the range of a few micrometers to several hundreds of micrometers) permits the reference electrode to be placed near the working electrode, which can significantly reduce the uncompensated ohmic drop across the working electrode and the

reference electrode. Furthermore, contamination of the testing solution is minimized owing to the lack of extra solvent ions, which may transfer from the electrolyte of the conventional Ag/AgCl reference electrodes to the testing solution. Good performances of solid-state thin-film Ag/AgCl reference electrodes fabricated by various approaches, such as sputtering [1-3], surface electroplating [4, 5], and screen printing [6, 7], have been reported.

Usually, the pre-deposited Ag layer is chlorinated by performing either electrochemical anodization in HCl solution or chemical oxidation in FeCl_3 solution to form a layer of AgCl. In contrast to adopting the conventional methods, the on-chip Ag/AgCl reference electrode of the proposed biomimetic chemical sensor is fabricated through direct evaporation of AgCl onto the sputtered Ag layer. The purpose of such fabrication method is to eliminate the possible contamination to the working and counter electrodes brought by the aggressive chloric solutions (e.g. HCl or FeCl_3). To evaluate the performance of the on-chip reference electrode, CV experiment with the redox couple of ferricyanide ions ($\text{Fe}(\text{CN})_6^{3-}$) and ferrocyanide ions ($\text{Fe}(\text{CN})_6^{4-}$) is used since it has been widely documented and studied [8-11]. The electrochemical reaction of the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ can be expressed as the redox reaction shown in Figure 4.1. If only the oxidized form ($\text{Fe}(\text{CN})_6^{3-}$) of a redox couple is initially present in the solution, the potential will be linearly scanned from a more positive one (initial potential) to a more negative one (switching potential) and then the scanning direction is shifted from the more negative one back to the more positive one (final potential). The waveform of the applied potential with respect to time in one cycle of CV is shown in Figure 4.2(a), where the negative-going potential sweep is denoted as “scan I” and the positive-going potential sweep is denoted as “scan II”. As illustrated in Figure 4.2(b), during the scan I, in which the potential is varied from the initial potential to the switching potential, $\text{Fe}(\text{CN})_6^{3-}$ ions will gain electrons to undergo the reduction reaction, producing a reduction

peak at a certain potential. While during the scan II, in which the potential is varied from the switching potential to the final potential, the previously generated Fe(CN)_6^{4-} ions will lose electrons to undergo the oxidation reaction, correspondingly producing an oxidation peak. The shape/current response of CV experiment actually derives from the continuous change of the concentration gradient in the vicinity of the working electrode surface. The magnitude of the current at peak potential (E_p) is termed as peak current (i_p), which can be calculated using the Randles–Sevcik equation if the redox reaction is only controlled by linear diffusion [12].

$$i_p = 0.4463 \frac{n^{3/2} F^{3/2}}{R^{1/2} T^{1/2}} D^{1/2} v^{1/2} AC \quad (1)$$

where i_p , peak current (A); n , number of electrons transferred; F , Faraday constant (C/mol); R , gas constant (V•C/mol•K); T , temperature (K); D , diffusion coefficient (cm^2/s); v , potential scan rate (V/s); A , electrode area (cm^2); C , bulk concentration (mol/cm^3).

Figure 4.1: Electrochemical reaction of the $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ redox couple.

Figure 4.2: (a) Schematic drawing to illustrate the waveform of the applied potential in one cycle of CV if only the oxidized form of a redox couple is initially present in the solution. (b) A representative cyclic voltammogram to show the peak potential (E_p) and the peak current

(i_p) of the reduction reaction, where the peak potential and the peak current of the oxidation reaction are not shown and can be obtained from the current response during the scan II.

Considering the reliable electrochemical performance of commercial electrodes, three commercial electrodes, i.e. commercial Au working electrode (CWE), commercial Ag/AgCl reference electrode (CRE) with 3 mol/L NaCl inner electrolyte, and commercial Pt counter electrode (CCE), were used to obtain reference data. Two types of fabricated on-chip reference electrodes (FREs), with one of them having a layer of evaporated AgCl coated on a sputtered Ag layer (FRE-Ag/AgCl) and the other one having only a layer of sputtered Ag (FRE-Ag), were investigated. The sensor base with different FREs was fabricated based on the same protocol (refer to section 3.3.1) and then packaged by connecting wires with one set of contact pads located on one side of the sensor base using conductive epoxy (purchased from Epoxy Technology, USA). The sensor base was placed in an oven to bake at 80 °C for 3 hrs. Once the sensor base cooled down to the room temperature, the resistance was carefully checked between one end of the wires and another set of contact pads located on the other side of the sensor base to ensure a stable connection was established. Thereafter, the non-conductive epoxy (purchased from Epoxy Technology, USA) was used to shield the surface of the sensor base except for the electrodes' area (which can be observed in Figure 4.3(b)). The sensor base was again put into an oven to bake at 80 °C for 1.5 hrs. Prior to the experiments, the sensor base was thoroughly cleaned by DI water. The CV experimental setup is shown in Figure 4.3(a), where three commercial electrodes were immersed in the testing solution of 0.002 mol/L potassium ferricyanide ($K_3[Fe(CN)_6]$) with 0.2 mol/L KCl supporting electrolyte. Subsequently, the same experiment was performed by only changing the CRE with the FRE, as shown in Figure 4.3(b). The entire electrochemical cell, which included all electrodes, was properly connected to a CHI 600C electrochemical workstation

via three crocodile clips. A compatible software was utilized to adjust the experimental parameters and control the operation of the electrochemical workstation.

Figure 4.3: Photograph of the experimental setup of the CV experiments with commercial Au working electrode (CWE), commercial Pt counter electrode (CCE), and (a) commercial Ag/AgCl reference electrode (CRE) and (b) fabricated on-chip reference electrode (FRE).

4.1.2 Stability over a range of scan rates

Three kinds of CV experiments were conducted in order to examine the MEMS-based thin-film reference electrodes. The first experiment was to check the stability of different reference electrodes over a range of scan rates. The CV experiments with the CRE were performed under an initial potential of 0.5 V, a final potential of 0.5 V, and a switching potential of -0.1 V. As for the FRE-Ag/AgCl and the FRE-Ag, experimental parameters were changed to an initial potential of 0.2 V, a final potential of 0.2 V, and a switching potential of -0.4 V. All the potentials were controlled and measured with respect to the corresponding reference electrodes. The scan rate of the CV experiments was varied from 0.01 to 0.25 V/s. The cyclic voltammograms of the CRE, FRE-Ag, and FRE-Ag/AgCl are shown in Figure 4.4(a), 4.4(b), and 4.4(c), respectively. It can be noticed that the CRE exhibits consistent reduction peak potentials around 0.17 V regardless of the change of the scan rate. Similarly, the reduction peak potentials for the FRE-Ag/AgCl also don't have a significant amount of drift except a small shift is observed when the scan rate is increased from 0.01 to 0.03 V/s.

All the potentials, on the other hand, are close to -0.12 V, which is more negative in comparison to the CRE. However, the movement of the reduction peak potentials of the FRE-Ag is somehow arbitrary, in which they initially move to the positive side at low scan rates (from 0.01 to 0.10 V/s) and then jump to the negative side at high scan rates (from 0.10 to 0.25 V/s). Such random potential drift is not desirable for a good reference electrode.

Figure 4.4: Comparisons of cyclic voltammograms under different scan rates from 0.01 to 0.25 V/s with (a) CRE, (b) FRE-Ag/AgCl, and (c) FRE-Ag. For (a), initial and final potential: 0.5 V; switching potential: -1.0 V. For (b) and (c), initial and final potential: 0.2 V; switching potential: -0.4 V. Potentials were measured with respect to (a) CRE, (b) FRE-Ag/AgCl, and (c) FRE-Ag.

4.1.3 Stability over a prolonged period of measurement

The second experiment was to determine the stability of the FRE-Ag/AgCl and the FRE-Ag over a prolonged period of measurement. With this intention in mind, the slowest scan rate of 0.01 V/s was selected for the CV experiments. For each reference electrode, seven runs of both negative-going and positive-going potential sweeps over 0.6 V were consecutively conducted, making the total experimental time to be 14 min. As shown in Figure 4.5(a), the FRE-Ag/AgCl displays almost identical cyclic voltammograms over the seven runs with a tiny amount of potential drift in the beginning of the experiment. However, there is an abrupt change of the reduction peak potentials in company with substantial distortion of the shape of the cyclic voltammograms among seven runs for the FRE-Ag, as shown in Figure 4.5(b). Specifically, the potentials shift from 0.08 V to -0.09 V during the first four runs. The change is around one-third of the total range of the potential sweep. This huge potential drift is unacceptable for a reference electrode. To test long-term stability of the FRE-Ag/AgCl, the potential variation of three sensors with FRE-Ag/AgCl was monitored in one hour and subsequently in eight consecutive hours. The experiments were conducted by measuring the potentials of the FRE-Ag/AgCl with respect to CRE in the solution of 0.2 mol/L KCl. The recorded results are shown in Figure 4.6(a) and 4.6(b), from which it can be observed that all three sensors with FRE-Ag/AgCl exhibit very little potential drift within one hour. The potential change for Sensor 1, 2, and 3 is around 2.0, 2.6, and 3.6 mV respectively, indicating a favorable stability of the FRE-Ag/AgCl. Within eight hours, the potential is decreased from 0.107 to 0.085 V for all three sensors with FRE-Ag/AgCl, which gives an average rate of potential drift about 2.8 mV/h. Such potential drift is expected since quasi/pseudo reference electrodes don't have thermodynamic equilibrium due to the absence of inner electrolyte, as explained in the previous section 4.1.1.

Figure 4.5: Comparisons of cyclic voltammograms with seven consecutive runs under scan rate of 0.01 V/s with (a) FRE-Ag/AgCl and (b) FRE-Ag. For (a), initial and final potential: 0.2 V; switching potential: -0.4 V. For (b), initial and final potential: 0.4 V; switching potential: -0.2 V. Potentials were measured with respect to (a) FRE-Ag/AgCl and (b) FRE-Ag.

Figure 4.6: Potential variation recorded in (a) one hour and (b) eight hours using three sensors with FRE-Ag/AgCl. Potentials were measured with respect to CRE.

4.1.4 Response consistency over multiple reference electrodes

The third experiment was intended to evaluate the consistency of the FRE-Ag/AgCl and the FRE-Ag. Six sensors of each type were used to carry out the CV experiments with same parameters including an initial potential of 0.2 V, a final potential of 0.2 V, a switching potential of -0.4 V, and a scan rate of 0.10 V/s. Experimental data for the FRE-Ag/AgCl are

illustrated in Figure 4.7(a), in which the average of the reduction peak potentials is about -0.12 V with a standard deviation of 0.01 V for six sensors. Figure 4.7(b) shows the response of six sensors with FRE-Ag, where the average of the reduction peak potentials is around -0.13 V, which is quite close to that of the FRE-Ag/AgCl. However, the standard deviation of the reduction peak potentials is around 0.1 V, which is ten times higher than that of the FRE-Ag/AgCl. According to the results obtained from the aforementioned experiments, it can be concluded that the FRE-Ag/AgCl fabricated by evaporating AgCl on a sputtered Ag layer is a good reference electrode to provide a stable reference point for the potential that is applied to the working electrode. The favorable performance of the FRE-Ag/AgCl is manifested by the repeatable response during long-time testing as well as the great consistency among different sensors. The FRE-Ag, however, seems to be a poor reference electrode due to the unstable outputs. The reason could be explained by the fact that without the coating of AgCl, the sputtered Ag layer gets contaminated in the air or during the measurement to form silver sulfide (Ag_2S) or some other compounds. The extent of the contamination may differ from each other, thereby inducing unpredictable fluctuations of the peak potentials.

Figure 4.7: Comparisons of the reduction peak potential of the CV experiments under scan rate of 0.10 V/s among six sensors with (a) FRE-Ag/AgCl and (b) FRE-Ag. Initial and final potential: 0.2 V; switching potential: -0.4 V. Potentials were measured with respect to (a) FRE-Ag/AgCl and (b) FRE-Ag. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

4.2 Structural characterization of micropillar electrode array

After evaluating the on-chip reference electrode, the micropillar working electrode array of the proposed biomimetic chemical sensor is specifically characterized from both material and electrochemical point of view. A good working electrode should be an ideal electrical conductor which allows fast and reproducible electron transfer across the interface between the electrode surface and the analyte solution. Therefore, upon fabrication, the electrical connection between two contact pads of each electrode was initially measured using a digital multimeter. Figure 4.8 displays the resistances of the bottom reference, working and counter electrodes of 20 sensors that were fabricated in one batch, where the resistance values for the majority of them are quite close each other except for the sensors ‘6’, ‘16’, and ‘20’. The resistances of 80 sensors that were fabricated in four different batches are listed in Table 4.1, where the average resistance values for the bottom reference, working, and counter electrodes are around 23, 16, and 10 Ω , respectively. The data regarding resistances of the remaining 60 sensors are presented in the Appendix A. If the resistance of any electrode of one sensor becomes 10% higher than the average value, that sensor is not used for experiments. For example, the sensors ‘6’, ‘16’, and ‘20’ show much higher resistances of all three electrodes compared with other ones and therefore are excluded for further investigations. A higher resistance usually suggests bad connectivity of the Au layer, which could be caused by several reasons, such as the stripping of the thin connection lines, the crack of the electrode or the contact pad, and the peeling of the Au layer at certain locations. Subsequently, the electrical connection of each micropillar with respect to the contact pad of the bottom working electrode was meticulously inspected under a high precision probe station, as shown in Figure 4.9. The probe station is equipped with a high-resolution optical microscope and two movable probes that can be finely tuned in three dimensions. A digital multimeter is connected to the two probes, in which the “Probe 1” is in contact with the micropillar and the

“Probe 2” is in contact with the contact pad. We found that the majority of the sensors made using the proposed fabrication protocol, as elaborated in section 3.3.2, had a good electrical connection of all the micropillars, indicating the feasibility of adopting MEMS technology to fabricate sensors with fine 3D structures.

Figure 4.8: The resistances of the bottom reference, working and counter electrodes of 20 sensors fabricated in one batch.

Table 4.1: Comparison of the resistances of 80 sensors fabricated in four batches

Batch No.	Reference electrode (Ω)	Working electrode (Ω)	Counter electrode (Ω)
1	23.3 ± 0.81	16.2 ± 0.64	10.3 ± 0.46
2	23.0 ± 0.74	16.0 ± 0.54	10.2 ± 0.40
3	23.6 ± 0.93	16.4 ± 0.63	10.4 ± 0.52
4	23.0 ± 0.85	16.0 ± 0.62	10.2 ± 0.43

Figure 4.9: Photograph with an enlarged view to show the setup of the electrical inspection of the micropillar working electrode array under a high precision probe station.

4.3 Electrochemical characterization of micropillar electrode array

Apart from being electrically conductive, a good working electrode should be able to capture the redox reaction that occurs in the vicinity of the electrode surface. To compare the performance of the micropillar working electrode array with that of the commercial disk (3 mm diameter) working electrode, commercial Ag/AgCl reference electrode (with 3 mol/L NaCl inner electrolyte) and commercial Pt counter electrode were used to conduct the CV experiments in order to minimize the possible bias. In the solution of 0.002 mol/L $\text{K}_3[\text{Fe}(\text{CN})_6]$ with 0.2 mol/L KCl, the CV experiments were carried out under the same condition, in which the initial potential, final potential, switching potential, and scan rate were set as 0.6 V, 0.6 V, -0.2 V and 0.05 V/s, respectively. A comparison of the cyclic voltammograms between the commercial electrode and the micropillar electrode array is shown in Figure 4.10(a), where clear reduction peak near the potential of 0.17 V and oxidation peak near the potential of 0.26 V for both electrodes are observed. On the other hand, the magnitudes of the peak currents exhibit a great amount of inequality, which is mainly caused by the difference of equivalent surface area between the commercial electrode and the micropillar electrode array. The cyclic voltammograms of four continuous scans for the commercial electrode and the micropillar electrode array are shown in Figure 4.10(b) and

4.10(c), respectively. Four voltammograms of the commercial electrode resemble almost identical shape, indicating high reproducibility of the electrode. Similarly, the micropillar electrode array also displays reproducible voltammograms among four scans with a tiny degree of disparity, which implies comparable performance of the micropillar electrode array to the commercial electrode.

Figure 4.10: (a) Comparison of the cyclic voltammogram between the commercial electrode (in red) and the micropillar electrode array (in black). The cyclic voltammograms of four continuous scans for (b) the commercial electrode and (c) the micropillar electrode array. Initial and final potential: 0.6 V; switching potential: -0.2 V; scan rate: 0.05 V/s. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

For the electrochemical reaction in which fast electron transfer occurs, the reaction rate (the resulting current) is determined by the mass transport of the electroactive materials to the

electrode-solution interface. There are three factors which will contribute to the mass transport, namely convection, migration, and diffusion [13-15]. As schematically illustrated in Figure 4.11, convection is the forced movement of the electroactive materials under the effect of an external perturbation, e.g. mechanical stirring. Migration is a movement of the charged particles induced by the effect of an electric field. Diffusion happens whenever there is a concentration gradient in the vicinity of the electrode surface. Since the CV experiments were conducted in the still solution without stirring, the convection factor didn't assist in the movement of the electroactive species, which was the $\text{Fe}(\text{CN})_6^{3-}$ ions in the experiments. In addition, the migration factor was also negligible as a sufficient quantity of supporting electrolyte, which was the KCl ions in the experiments, was added into the testing solution. Hence, during the CV experiments, the diffusion factor was the only effect involved to transport the $\text{Fe}(\text{CN})_6^{3-}$ ions to the working electrode. Recalling from equation (1), as mentioned in section 4.1.1, the peak current of CV experiments for a given redox reaction should be linearly related to the square root of the scan rate. Figure 4.12(a) depicts a series of cyclic voltammograms for the micropillar electrode array when only the scan rate was varied from 0.01 to 0.15 V/s. Legible and undistorted reduction and oxidation peaks can be easily distinguished among the voltammograms. Figure 4.12(b) shows the fitting plot of the magnitude of the reduction peak current with respect to the square root of the scan rate, in which a high linearity ($R^2 = 0.998$) is observed, demonstrating the capability of the micropillar electrode array to precisely register the redox reaction. According to the aforementioned discussions, the proposed micropillar electrode array of the biomimetic chemical sensor could serve as a good working electrode to be used for other electrochemical measurements.

Figure 4.11: Schematic representation of the three factors, namely convection, migration, and diffusion, which contribute to the mass transport of the electroactive materials to the electrode-solution interface [15].

Figure 4.12: (a) Cyclic voltammograms for the micropillar electrode array under different scan rates from 0.01 to 0.15 V/s. (b) The fitting plot of the magnitude of the reduction peak current versus the square root of the scan rate. Initial and final potential: 0.6 V; switching potential: -0.2 V. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

In order to estimate the Au coverage area on all the micropillars, the CV responses of the micropillar electrode array are compared with that of the commercial electrode based on the equation (1) presented in section 4.1.1. Besides, the Au coverage area on the sensor without micropillar electrode array is also evaluated. All the detailed calculations are provided in the Appendix B. Given the surface area of the commercial electrode (3 mm diameter disk) is 7.07 mm^2 , the Au coverage area on the sensor with and without micropillar electrode array are estimated as 3.04 ± 0.03 and $2.33 \pm 0.02 \text{ mm}^2$, respectively. By extending the 2D electrode into a 3D micropillar electrode array, the total sensing area of the working electrode is greatly increased by 30 percent. Assuming all the micropillars have identical cylindrical shape, the theoretical surface area of the micropillar electrode array calculated based on the electrode dimension, as shown in Figure 4.13, is about 2.87 mm^2 , where the calculation is illustrated in equation (2). The estimated value (3.04 mm^2) is about 6% larger than the theoretical one (2.87 mm^2), which basically means the micropillar electrode array is fully covered by Au. Therefore, it demonstrates that employing the proposed MEMS techniques to fabricate the micropillar electrode array, as described in section 3.3.2, is quite successful. The extra surface area could be due to the small enlargement of the top section of the micropillars during the fabrication, as the photolithography was performed by firstly exposing the UV light to the top surface of the thick SU-8 photoresist. Similar diffraction effect is also reported in other literature [16-18].

Figure 4.13: Dimension of the bottom working electrode together with the micropillar electrode array, where only half of 24 micropillars are shown in the drawing in order to improve the clarity.

Theoretical surface area of the micropillar electrode array (2)

= surface area of the bottom semi-annulus – bottom surface area of the 24 micropillars

+ top surface area of the 24 micropillars + side surface area of the 24 micropillars

$$= 0.5 \times (\pi \times 1.5^2 - \pi \times 1^2) - 24 \times \pi \times 0.05^2 + 24 \times \pi \times 0.05^2 + 24 \times \pi \times$$

$$0.1 \times 0.12$$

$$= 2.87 \text{ mm}^2$$

References

- [1] Suzuki, H., Hiratsuka, A., & Sasaki, S. et al. (1998). Problems associated with the thin-film Ag/AgCl reference electrode and a novel structure with improved durability. *Sensors and Actuators B: Chemical*, 46(2), 104-113.
- [2] Matsumoto, T., Ohashi, A., & Ito, N. (2002). Development of a micro-planar Ag/AgCl quasi-reference electrode with long-term stability for an amperometric glucose sensor. *Analytica Chimica Acta*, 462(2), 253-259.
- [3] Kim, T. Y., Hong, S. A., & Yang, S. (2015). A solid-state thin-film Ag/AgCl reference electrode coated with graphene oxide and its use in a pH sensor. *Sensors*, 15(3), 6469-6482.
- [4] Polk, B. J., Stelzenmuller, A., & Mijares, G. et al. (2006). Ag/AgCl microelectrodes with improved stability for microfluidics. *Sensors and Actuators B: Chemical*, 114(1), 239-247.
- [5] Almeida, F. L., Fontes, M. B. A., & Jimenez, C. et al. (2008). Secondary Ag/AgCl pseudo-reference electrode on silicon substrate. *ECS Transactions*, 14(1), 73-82.
- [6] Simonis, A., Krings, T., & Luth, H. et al. (2001). A “hybrid” thin-film pH sensor with integrated thick-film reference. *Sensors*, 1(6), 183-192.
- [7] da Silva, E. T. S. G., Miserere, S., & Kubota, L. T. et al. (2014). Simple on-plastic/paper inkjet-printed solid-state Ag/AgCl pseudoreference electrode. *Analytical Chemistry*, 86(21), 10531-10534.
- [8] Gomathi, H., & Rao, G. P. (1990). Simple electrochemical immobilization of the ferro/ferricyanide redox couple on carbon electrodes. *Journal of Applied Electrochemistry*, 20(3), 454-456.
- [9] Beriet, C., & Pletcher, D. (1993). A microelectrode study of the mechanism and kinetics of the ferro/ferricyanide couple in aqueous media: the influence of the electrolyte and its concentration. *Journal of Electroanalytical Chemistry*, 361(1-2), 93-101.

- [10] Osolovitch, J., Li, Y. J., & Donner, C. et al. (2003). The $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ charge transfer reaction on Au(111) revisited in the presence and absence of a two-dimensional, condensed organic film. *Journal of Electroanalytical Chemistry*, 541, 163-174.
- [11] Emery, S. B., Hubble, J. L., & Roy, D. (2005). Time resolved impedance spectroscopy as a probe of electrochemical kinetics: the ferro/ferricyanide redox reaction in the presence of anion adsorption on thin film gold. *Electrochimica Acta*, 50(28), 5659-5672.
- [12] Monk, P. M. S. (2001). *Fundamentals of electroanalytical chemistry*. Chichester, NY: Wiley.
- [13] Wang, J. (2006). *Analytical electrochemistry* (3rd ed.). Hoboken, NJ: Wiley-VCH.
- [14] Zoski, C. G. (2007). *Handbook of electrochemistry*. Amsterdam, Boston: Elsevier.
- [15] Moretto, L. M., & Seeber, R. (2014). Controlled potential techniques in amperometric sensing. In L. M. Moretto, & K. Kalcher (Eds.), *Environmental analysis by electrochemical sensors and biosensors* (pp. 239-282). New York, NY: Springer.
- [16] Chuang, Y. J., Tseng, F. G., & Lin, W. K. (2002). Reduction of diffraction effect of UV exposure on SU-8 negative thick photoresist by air gap elimination. *Microsystem Technologies*, 8(4), 308-313.
- [17] Lawes, R. A. (2005). Manufacturing tolerances for UV LIGA using SU-8 resist. *Journal of Micromechanics and Microengineering*, 15(11), 2198-2203.
- [18] Iliescu, C., Taylor, H., & Avram, M. et al. (2012). A practical guide for the fabrication of microfluidic devices using glass and silicon. *Biomicrofluidics*, 6(1), 1-16.

5

LEAD DETECTION USING BIOMIMETIC CHEMICAL SENSOR

This chapter provides a comprehensive assessment of the biomimetic chemical sensor towards lead detection. Parameters such as the potential window, deposition potential, and deposition time, which will affect the performance of the sensor, are studied. In particular, the effect of flow rate on the biomimetic chemical sensor without and with micropillar electrode array is inspected. The possible reasons that could induce the interesting experimental observations are explained, through which the proposed hypotheses are verified. The last section presented in this chapter documents the analytical performance of the biomimetic chemical sensor, including the sensitivity, linear range as well as limit of detection.

5.1 Chemicals, apparatus and measurement procedures

The chemicals and reagents used in the following experiments were of analytical grade. The DI water with an electrical resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ was collected from a Milli-Q system (Millipore, Singapore) and used to prepare all the solutions. The Bi standard stock solution with a concentration of 1000 mg/L was purchased from Merck, Singapore and diluted accordingly. The Pb standard stock solution with a concentration of 1000 mg/L was purchased from Sigma-Aldrich, Singapore and also diluted during the experiments. The reason to use Bi solution in the experiments is that Bi ions tend to form a low-temperature alloy with Pb ions. This will significantly contribute to the accumulation of Pb ions on the working electrode, as demonstrated by Wang et al. [1], Kefala et al. [2], de-Carvalho et al. [3], Cesarino et al. [4], etc. The acetate buffer with a pH value of 4.6 was purchased from Sigma-Aldrich, Singapore and used as the supporting electrolyte after dilution (0.1 mol/L). Selecting this supporting electrolyte is based on the consideration that acetate buffer is the most widely used medium for the measurement of Pb ions when in-situ plated BFE is involved [5]. Favorable quantification of Pb ions using acetate buffer supporting electrolyte has been reported by several researchers [1, 2, 4, 6-8]. The experimental setup is shown in Figure 5.1(a) and 5.1(b), in which the testing solution was stored in a glass vial (Alpha Analytical, Singapore). A Teflon cap (Alpha Analytical, Singapore) with small holes was tightly attached to the glass vial to prevent the possible contamination of large particles from the surroundings. The glass vial, Teflon cap were thoroughly cleaned by DI water before the experiments. A Teflon tube (Alpha Analytical, Singapore) was used to deliver the testing solution with one end being inserted into the testing solution and the other end being linked to the inlet of the microfluidic channel. A high precision dispenser purchased from Atome Sciences, Singapore was used to pump the testing solution with specified flow rate. The contact pads of the three electrodes, i.e. reference, working and counter electrodes, were connected to a CHI 600C

electrochemical workstation (CH Instruments, USA) through crocodile clips. A computer was connected to the electrochemical workstation, through which the experimental parameters were adjusted and the measurements were controlled.

Figure 5.1: (a) Photograph of the experimental setup for testing the biomimetic chemical sensor. (b) Photograph of an enlarged view to show the configuration.

CV experiments were performed by firstly dispensing 0.1 mol/L acetate buffer solution into the microfluidic channel and then the dispenser was switched off. After that, the measurements with an initial potential of -1.0 V, a final potential of 0.6 V, and a scan rate of 0.05 V/s were recorded under quiescent condition. While SWASV experiments were carried

out in the solution of 300 $\mu\text{g/L}$ Bi ions together with different concentrations of Pb ions by initially applying a deposition potential of -0.8 V (after optimization) to the working electrode for 30 s (after optimization) to accumulate target metal ions. The testing solution was continuously dispensed into the microfluidic channel during the deposition step. Subsequently, the dispenser was switched off and a 5 s quiescent time was provided. The voltammograms were recorded in the potential range of -0.8 to 0 V with a frequency of 50 Hz, an amplitude of 0.05 V, and a step potential of 0.005 V. Ahead of next measurement, a conditioning potential of 0 V was applied to the working electrode for 120 s with continuous dispensing in order to refresh the electrode surface. The potentials controlled and measured during the experiments were with respect to the on-chip Ag/AgCl reference electrode. All the experiments were conducted at room temperature and all the solutions used were not purged. Prior to the experiments, the flow rate of the high precision dispenser was calibrated using the DI water when the knob was turned at different positions. The results are given in Figure 5.2, where the flow rate gradually increases as the knob is turned from position '1' to position '10'. However, the relationship is not linear and large deviation of the flow rate is obtained at position '8', '9', and '10'. Therefore, position '1', '4', '5', '6', and '7' are selected for the experiment when the effect of flow rate on the response of the sensor is studied. Position '2' and position '3' are excluded from the experiment since the flow rates at these two positions are not clearly distinguishable to the one at position '1'.

Figure 5.2: Calibration of the high precision dispenser with DI water. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

5.2 Working range of the sensor

For an electrochemical sensor, the potential window is an important parameter to study since it defines the range of heavy metal ions that can be determined using the sensor. The potential window of the biomimetic chemical sensor was investigated by performing CV experiments in the solution of 0.1 mol/L acetate buffer. Figure 5.3(a) depicts the responses of three sensors, in which the negative and positive limits of the sensor are around -0.8 and 0.4 V with respect to the on-chip Ag/AgCl reference electrode, respectively. A significantly high negative current is observed when the potential becomes lower than -0.8 V, which is mainly caused by the reduction of hydrogen ions in the solution to generate hydrogen bubbles. On the other end, a positive current appears more prominent when the potential becomes higher than 0.4 V. This is possible due to the oxidation of the Au metal layer, which will induce fast stripping and further damage of the working electrode if the potential is held at this level for long period of time. A preliminary SWASV experiment was initially performed in the solution of 30 μg/L Pb ions with 0.1 mol/L acetate buffer. Figure 5.3(b) illustrates the voltammogram recorded, in which a legible stripping peak around the potential of -0.57 V is

observed, suggesting that the biomimetic chemical sensor is applicable for Pb detection. In addition, the current begins to gradually increase again when the potential is more positive than -0.18 V, which corresponds to the stripping of Bi metal layer formed during the deposition step. As mentioned in section 5.1, the deposition process of all the SWASV experiments presented in this study is conducted by simultaneously accumulating Bi ions together with target Pb ions.

Figure 5.3: (a) Investigation of potential window of three biomimetic chemical sensors in the solution of 0.1 mol/L acetate buffer. Initial potential: -1.0 V; final potential: 0.6 V; scan rate: 0.05 V/s. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode. (b) The anodic stripping voltammogram recorded in the solution of 30 μg/L Pb ions with 0.1

mol/L acetate buffer. Deposition potential: -0.7 V; deposition time: 40 s; flow rate: 0.25 $\mu\text{L/s}$. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode.

5.3 Influence of deposition potential

Since the accumulation of target metal ions to the working electrode during the deposition step is directly related to two parameters, i.e. deposition potential and deposition time, it is necessary to search for the most favorable value of the parameters in order to optimize the performance of the sensor. The effect of deposition potential on the stripping peak current of the biomimetic chemical sensor was investigated in the solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. Under fixed deposition time of 40 s and fixed flow rate of 0.25 $\mu\text{L/s}$, there is a significant increase of the peak current when the deposition potential is varied from -0.5 to -0.8 V, as shown in Figure 5.4. This is possibly due to the fact that more ions are involved in the accumulation process when more negative potential (higher energy) is provided. Once the deposition potential crosses over -0.8 V, the peak current begins to gradually decrease, which is possibly associated with the generation of hydrogen bubbles in the solution. The bubbles may partially block the surface of the working electrode, resulting in reduced amount of metal ions that could participate in the accumulation process. The evolution of hydrogen bubbles can also be supported by the observation from the CV experiments, as illustrated in Figure 5.3(a). This phenomenon was reported by several researchers [9-12]. At the deposition potential of -1.1 V, the stripping peak current increases again, which is possibly related to the nucleation of metal ions at different sites during the deposition step. When the deposition potential is applied at moderately negative values (between -0.8 and -1.0 V), the nucleation of metal ions only occurs on the thermodynamically privileged sites of the working electrode [13], so does the evolution of hydrogen bubbles. When the deposition potential is applied at very negative values (more negative than -1.0 V), the nucleation of metal ions will also occur on the thermodynamically non-privileged sites of

the working electrode [13]. However, hydrogen evolution still happens on those privileged sites of the working electrode since the bubbles tend to become bigger at more negative potentials. Such difference could possibly cause the increase of the stripping peak current. This trend was also observed by Krolicka et al. [13]. To prevent the hydrogen evolution, the deposition potential of -0.8 V was selected for the remaining experiments.

Figure 5.4: Effect of deposition potential on the stripping peak current of the biomimetic chemical sensor in the solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. Deposition time: 40 s; flow rate: 0.25 $\mu\text{L/s}$. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

5.4 Influence of deposition time

The effect of deposition time on the stripping peak current of the biomimetic chemical sensor was investigated in the same solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. Under the optimized deposition potential of -0.8 V and unchanged flow rate of 0.25 $\mu\text{L/s}$, the deposition time was changed between 10 and 70 s. As shown in Figure 5.5, the peak current is initially increased when the deposition time is extended to 30 s, which could be caused by the fact that more metal ions are brought to the vicinity of the working electrode with longer

dispensing time as the accumulation process is performed in a flowing manner. Subsequently, a relatively constant peak current is observed, after which the peak current starts to drop. This phenomenon could result from the possible saturation effect, in which prolonged deposition time is not helpful for a further increment of the stripping current. Wang et al. [11], Figueiredo-Filho et al. [14], and Wan et al. [15] also reported a similar observation. To circumvent the quick saturation of the working electrode especially in the solutions with high concentration, the deposition time of 30 s was selected for the remaining experiments.

Figure 5.5: Effect of deposition time on the stripping peak current of the biomimetic chemical sensor in the solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. Deposition potential: -0.8 V; flow rate: 0.25 $\mu\text{L/s}$. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

5.5 Influence of flow rate

After optimizing the deposition potential and deposition time, the effect of flow rate on the stripping peak current for the biomimetic chemical sensor with and without micropillar electrode array was investigated in the solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. As mentioned in section 5.1, five different flow rates, namely 0.15, 0.25, 0.35, 0.45,

and 0.64 $\mu\text{L/s}$ which correspond to the knob position '1', '4', '5', '6', and '7', were used in the experiments. As shown in Figure 5.6(a), the peak current of the biomimetic chemical sensor with micropillar electrode array almost linearly increases when the testing solution is dispensed at a higher flow rate. As for the biomimetic chemical sensor without micropillar electrode array, however, the peak current gradually decreases when the flow rate is increased except for the last flow rate, in which a tiny rise is observed. The ratio of the stripping peak current for the biomimetic chemical sensor with micropillar electrode array over the one without micropillar electrode array is displayed in Figure 5.6(b), where the current increase becomes larger from 73.3% up to 255.4% when the flow rate is escalated from 0.15 to 0.45 $\mu\text{L/s}$. The current increase slightly reduces to 250% when the flow rate is set as 0.64 $\mu\text{L/s}$. Referring to the estimation of the Au coverage on the working electrode for both sensors presented in section 4.3, an approximate 30 percent enlargement of the total sensing area for the biomimetic chemical sensor with micropillar electrode array in comparison to the one without micropillar electrode array. Therefore, under same measurement condition, the current increase between the biomimetic chemical sensor with and without micropillar electrode array should be also around 30 percent. However, the experimental results suggest that the disparity of the total sensing area is not the only contributor to the current increase.

Figure 5.6: (a) Effect of flow rate on the stripping peak current between the biomimetic chemical sensor with and without micropillar electrode array in the solution of 30 $\mu\text{g/L}$ Pb ions with 0.1 mol/L acetate buffer. (b) The ratio of the stripping peak current between the biomimetic chemical sensor with and without micropillar electrode array. Deposition potential: -0.8 V; deposition time: 30 s. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

To elucidate this exhilarating finding, one can compare the ion collection efficiency between the biomimetic chemical sensor without and with micropillar electrode array. For the sensor without micropillar electrode array, the bottom thin-film working electrode is at least

partially immersed inside the stagnation/boundary layer of the flowing solution. The target metal ions dissolved in the solution can only be transported to the vicinity of the working electrode under the influence of diffusion effect. High flow rate could not greatly enhance the ion collection efficiency of the thin-film working electrode as diffusion is only driven by the concentration gradient. Besides, the velocity profile of laminar flow/solution along the transverse plane (which is parallel to the bottom working electrode) has a parabolic shape, which means the majority of the solution will pass through the middle section of the working electrode. Hence, as the flow rate increases, the electrode area that is in contact with the flowing solution is diminished, which results in less amount of metal ions to be adsorbed on the electrode surface during the same period of deposition time, thereby leading to the decline of the stripping peak current. The tiny rise of the peak current could be possibly due to the thinner boundary layer developed at a very high flow rate, which, to some extent, compensates for the reduced available area of the working electrode. However, for the sensor with micropillar electrode array, the 3D structure allows all individual micropillar electrodes to extend from the boundary layer of the flowing solution. Under such condition, the convection effect will dominate the mass transport of the target metal ions to the vicinity of the electrode. Consequently, the higher flow rate can induce stronger convection effect, bringing more metal ions to the micropillar electrodes, which in turn elevates the stripping peak current. In addition, the arrangement of the electrode array forms a multi-channel system by neighboring micropillars, just like the gap system inside the olfactory rosette of the lemon shark, which guides the solution to move through the micropillars, ensuring sufficient available electrode area for ion accumulation even at higher flow rate. Therefore, the ion collection efficiency for the sensor with micropillar electrode array can be significantly enhanced. Based on the above discussions, it can be encapsulated that the current increase is attributed to the enlargement of the total sensing area as well as the interaction between the

flowing solution and the micropillar electrode array. In consideration of both satisfactory sensitivity and possible saturation, the flow rate of 0.35 $\mu\text{L/s}$ was selected for the remaining experiments.

5.6 Analytical performance of the sensor

Utilizing the optimized experimental parameters, including a deposition potential of -0.8 V, a deposition time of 30 s, and a flow rate of 0.35 $\mu\text{L/s}$, the analytical performance of the biomimetic chemical sensor towards Pb detection was investigated by performing a series of SWASV experiments with increased concentration of Pb ions. The anodic stripping voltammograms recorded are shown in Figure 5.7(a), in which undistorted and legible stripping peaks located near the potential of -0.57 V can be distinguished. The relationship between the stripping peak currents with respect to different concentrations of Pb ions from 1 to 130 $\mu\text{g/L}$ is shown in Figure 5.7(b), where high linearity ($R^2 = 0.999$) of the calibration plot is obtained. The sensor exhibits an analytical sensitivity of 32 $\text{nA}/(\mu\text{g/L})$ under a very short deposition time of 30 s. Figure 5.7(c) depicts the LOD of the biomimetic chemical sensor, which is recorded about 0.2 ppb. The LOD was determined by slowly increasing the concentration of Pb ions in the testing solution until a clear stripping voltammogram with a signal-to-noise ratio of at least 3 was observed.

Figure 5.7: (a) A series of anodic stripping voltammograms recorded for the biomimetic chemical sensor with increased concentration of Pb ions from 1 to 130 $\mu\text{g/L}$. (b) Corresponding calibration plot of the stripping peak current with respect to the concentration of Pb ions. (c) The LOD of the sensor recorded in the solution of 0.2 ppb Pb ions with 0.1 mol/L acetate buffer. Deposition potential: -0.8 V; deposition time: 30 s; flow rate: 0.35 $\mu\text{L/s}$. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode.

A comparison pertaining to the deposition mode, deposition time, and LOD between the proposed biomimetic chemical sensor and other solid-state chemical sensors fabricated using either SPE or MEMS technique is listed in Table 5.1. For all the sensors, the Pb detection is based on the SWASV electrochemical method and the sensing material of the working electrode is related to different forms of Bi. It can be noticed that the deposition time required to identify low-level Pb ions for the biomimetic chemical sensor is much shorter than other sensors. The fast detection capability of the biomimetic chemical sensor is highly contributed by the structural configuration of the supporting electrode. The approach of stretching a planar electrode to a 3D electrode array not only reduces the footprint that is needed to place the supporting electrode, but also renders exceptionally enhanced ion collection efficiency to the sensor. Without the employment of forced mechanical perturbation/stirring to the testing solution, the proposed biomimetic chemical sensor manifests promising application for on-site, quick, and accurate trace Pb determination. However, in order to make the proposed sensor to be used for real-life environmental application, a comprehensive analysis of the sensor response in the aspects of possible matrix effects associated with other heavy metal ions and/or organic pollutants present in the water at testing locations should be taken into consideration. Moreover, water properties, e.g. salinity, acidity, alkalinity, hardness, etc., may also have some influences on the sensor. For instance, water samples having a slightly acidic condition (pH 4 to 5) are preferable for the proposed chemical sensor to avoid hydrolysis of non-complexed Bi ions since Pb and Bi ions are co-deposited onto the working electrode during the deposition process.

Table 5.1: Comparison of different solid-state chemical sensors for Pb detection

Working electrode	Supporting electrode	Deposition mode	Deposition time (s)	LOD ($\mu\text{g/L}$)	Reference
In-situ plated Bi film	Micropillar array (1.96 mm^2)	Flowing ($0.35 \mu\text{L/s}$)	30	0.2	This work
Synthesized Bi nanoparticles	Planar disk (12.57 mm^2)	Stirring (500 rpm)	120	1.3	Rico et al. [16]
Synthesized Bi nanoparticles	Planar disk (12.57 mm^2)	Flowing ($38.3 \mu\text{L/s}$)	120	0.9	Rico et al. [16]
In-situ plated Bi film	Planar disk (7.07 mm^2)	Stirring ($\text{NA}^\#$)	300	0.15	Quintana et al. [17]
Bi nanoparticles with porous carbon	Planar disk (12.57 mm^2)	Stirring (700 rpm)	300	2.3	Niu et al. [18]
Electrochemically reduced Bi_2O_3	Planar disk (7.07 mm^2)	Quiescent	120	5.0	Kadara et al. [19]
In-situ plated Bi film	Planar microband (4.57 mm^2)	Wicking	120	1.0	Nie et al. [20]
In-situ plated Bi film	Planar disk (3.14 mm^2)	Quiescent	120	2.0	Tan et al. [21]
Evaporated Bi film	Planar microband (3 mm^2)	Quiescent	60	8.0	Zou et al. [22]
Sputtered Bi film	Planar microdisk array (25 mm^2)	Quiescent	120	0.6	Kokkinos et al. [23]

[#]NA stands for ‘not available’. The number in the bracket under the column of “supporting electrode” indicates the area of the footprint.

References

- [1] Wang, J., Lu, J. M., & Hocevar, S. B. et al. (2000). Bismuth-coated carbon electrodes for anodic stripping voltammetry. *Analytical Chemistry*, 72(14), 3218-3222.
- [2] Kefala, G., Economou, A., & Voulgaropoulos, A. et al. (2003). A study of bismuth-film electrodes for the detection of trace metals by anodic stripping voltammetry and their application to the determination of Pb and Zn in tapwater and human hair. *Talanta*, 61(5), 603-610.
- [3] de-Carvalho, L. M., do-Nascimento, P. C., & Koschinsky, A. et al. (2007). Simultaneous determination of cadmium, lead, copper, and thallium in highly saline samples by anodic stripping voltammetry (ASV) using mercury-film and bismuth-film electrodes. *Electroanalysis*, 19(16), 1719-1726.
- [4] Cesarino, I., Gouveia-Caridade, C., & Pauliukaite, R. et al. (2010). Characterization and application of bismuth-film modified graphite-polyurethane composite electrodes. *Electroanalysis*, 22(13), 1437-1445.
- [5] Arduini, F., Calvo, J. Q., & Amine, A. et al. (2010). Bismuth-modified electrodes for lead detection. *Trends in Analytical Chemistry*, 29(11), 1295-1304.
- [6] Hocevar, S. B., Wang, J., & Deo, R. P. et al. (2002). Potentiometric stripping analysis at bismuth-film electrode. *Electroanalysis*, 14(2), 112-115.
- [7] Kachoosangi, R. T., Banks, C. E., & Ji, X. et al. (2007). Electroanalytical determination of cadmium(II) and lead(II) using an in-situ bismuth film modified edge plane pyrolytic graphite electrode. *Analytical Sciences*, 23(3), 283-289.
- [8] Li, Z., Chen, L., & He, F. et al. (2014). Square wave anodic stripping voltammetric determination of Cd^{2+} and Pb^{2+} at bismuth-film electrode modified with electroreduced graphene oxide-supported thiolated thionine. *Talanta*, 122, 285-292.

- [9] Chen, C., Niu, X., & Chai, Y. et al. (2013). Determination of lead(II) using screen-printed bismuth-antimony film electrode. *Electroanalysis*, 25(6), 1446-1452.
- [10] Yang, D., Wang, L., & Chen, Z. et al. (2014). Voltammetric determination of lead (II) and cadmium (II) using a bismuth film electrode modified with mesoporous silica nanoparticles. *Electrochimica Acta*, 132, 223-229.
- [11] Wang, Z., Wang, H., & Zhang, Z. et al. (2014). Electrochemical determination of lead and cadmium in rice by a disposable bismuth/electrochemically reduced graphene/ionic liquid composite modified screen-printed electrode. *Sensors and Actuators B: Chemical*, 199, 7-14.
- [12] Xu, Y., Zhang, W., & Shi, J. et al. (2016). Microfabricated interdigitated Au electrode for voltammetric determination of lead and cadmium in Chinese mitten crab (*Eriocheir sinensis*). *Food Chemistry*, 201, 190-196.
- [13] Krolicka, A., Bobrowski, A., & Kowal, A. (2006). Effects of electroplating variables on the voltammetric properties of bismuth deposits plated potentiostatically. *Electroanalysis*, 18(17), 1649-1657.
- [14] Figueiredo-Filho, L. C. S., Janegitz, B. C., & Fatibelo-Filho, O. et al. (2013). Inexpensive and disposable copper mini-sensor modified with bismuth for lead and cadmium determination using square-wave anodic stripping voltammetry. *Analytical Methods*, 5(1), 202-207.
- [15] Wan, H., Sun, Q., & Li, H. et al. (2015). Screen-printed gold electrode with gold nanoparticles modification for simultaneous electrochemical determination of lead and copper. *Sensors and Actuators B: Chemical*, 209, 336-342.
- [16] Rico, M. A. G., Olivares-Marin, M., & Gil, E. P. (2009). Modification of carbon screen-printed electrodes by adsorption of chemically synthesized Bi nanoparticles for the voltammetric stripping detection of Zn(II), Cd(II) and Pb(II). *Talanta*, 80(2), 631-635.

- [17] Quintana, J. C., Arduini, F., & Arnine, A. et al. (2011). Part I: A comparative study of bismuth-modified screen-printed electrodes for lead detection. *Analytica Chimica Acta*, 707(1), 171-177.
- [18] Niu, P., Fernandez-Sanchez, C., & Gich, M. et al. (2016). Screen-printed electrodes made of a bismuth nanoparticle porous carbon nanocomposite applied to the determination of heavy metal ions. *Microchimica Acta*, 183(2), 617-623.
- [19] Kadara, R. O., Jenkinson, N., & Banks, C. E. (2009). Disposable bismuth oxide screen printed electrodes for the high throughput screening of heavy metals. *Electroanalysis*, 21(22), 2410-2414.
- [20] Nie, Z., Nijhuis, C. A., & Gong, J. et al. (2010). Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip*, 10(4), 477-483.
- [21] Tan, S. N., Ge, L., & Wang, W. (2010). Paper disk on screen printed electrode for one-step sensing with an internal standard. *Analytical Chemistry*, 82(21), 8844-8847.
- [22] Zou, Z., Jang, A., & MacKnight, E. et al. (2008). Environmentally friendly disposable sensors with microfabricated on-chip planar bismuth electrode for in situ heavy metal ions measurement. *Sensors and Actuators B: Chemical*, 134(1), 18-24.
- [23] Kokkinos, C., Economou, A., Raptis, I. (2012). Microfabricated disposable lab-on-a-chip sensors with integrated bismuth microelectrode arrays for voltammetric determination of trace metals. *Analytica Chimica Acta*, 710, 1-8.

6

ZINC DETECTION USING FLEXIBLE LCP CHEMICAL SENSOR

This chapter details out the design of a miniaturized and compact chemical sensor for zinc detection constructed on a novel robust material, liquid-crystal polymer (LCP). Incorporation of the LCP substrate offers improved reliability, high durability and flexibility to the sensor, rendering it surface-mountable feature for in-situ deployment. The fabrication procedures of the sensor are provided and sensing capability of the sensor is optimized in aspects of the potential window, deposition potential, and deposition time. Reproducibility, interference, and analytical performance of the sensor are also investigated. Real-time applicability of the sensor is corroborated by performing sea trial testing with a sensor array attached to the hull of a remotely-controlled kayak.

6.1 Background and motivation

Zn, one of the most common water pollutants, is widely used in several industries, such as galvanization, petroleum, lubrication, pigmentation, alloy manufacturing, and battery production. Zn metal ions can easily get into the groundwater through discharge of untreated industrial wastewater and excessive fertilization in the Zn-deficient farmlands. Although the recommended value of Zn ions in drinking water, as provided by WHO, is relatively high (3 mg/L) [1], over-intake will also induce severe gastrointestinal problems, hair loss, muscle spasms and sciatica [2]. Conventional water quality monitoring requires water samples to be manually collected and subsequently analyzed in the centralized laboratory. Analytical techniques, such as atomic absorption spectrometry (AAS) [3, 4], inductively coupled plasma atomic emission spectrometry (ICP-AES) [5, 6], and inductively coupled plasma mass spectrometry (ICP-MS) [7, 8], are usually adopted to determine the concentration of target metal ions. Despite these approaches are sensitive to quantify the water quality, their complicated operation, high instrumentation cost, and bulky size limit the application for in-situ measurement. Real-time data regarding the level of target metal ions in the concerned areas cannot be attained by using the aforementioned techniques.

To circumvent the complexity of conventional testing techniques, researchers have started to use electroanalytical methods, especially ASV due to the simplicity of experimental arrangement, low cost, and favorable sensitivity. The material of the working electrode is the most critical sensing element of ASV to ensure successful voltammetric analysis. Over the last decade, BFEs have gained wide acceptance because of their non-toxic property, making them potential alternatives to replace mercury electrodes. Apart from being environmentally friendly, BFEs also offer a number of attractive stripping merits, such as reproducible voltammetric response, easily distinguishable stripping peaks, more negative potential

window and high signal-to-background ratio [9]. Research pertaining to the development of simple-to-use, inexpensive, and compact sensors with BFEs that can be used for point-of-care/in-situ detection is of great interest to both academics and industries. Among all the attempts, electrochemical sensors having ex-situ prepared BFEs with thin-film reference and counter electrodes provide a promising candidature for possible in-situ quantification of heavy metal ions. This is mainly attributed to the possibility to integrate all necessary sensing and accessory components onto a single device.

In order to achieve long-term in-situ measurement, the material of the sensor substrate also plays a critical role in maintaining operational reliability as unremitting subjection to rigorous surroundings is inevitable. Therefore, the most desired properties of such material include strong mechanical stability, low moisture absorption, superior hermetic sealing, and chemical inertness. We have chosen liquid-crystal polymer (LCP) material to form the substrate of the MEMS chemical sensor due to its compliance with the aforementioned features. LCP, made up of aligned molecule chains with crystal-like spatial regularity, is a class of aromatic thermoplastic polymers and demonstrates a highly ordered structural configuration in the solid state. LCP material improves the operational reliability of the sensors since it has higher fracture strength as compared to other materials such as plastic, cloth, paper, silicon, and glass, enabling chemical sensors with LCP substrate to be deployed for harsh environmental applications. LCP also demonstrates high weatherability in the aqueous media due to its extremely low moisture absorption ($\sim 0.02\%$) [10] even after extended periods of exposure to the media. LCP is exceptionally inert and does not suffer attacks from various corrosive chemicals that can easily damage materials such as plastic, paper, and silicon.

LCP can also maintain stable dimensional properties in the fluid environment due to the low permeability of the material to water. In addition, the flexibility of LCP material allows it to be surface-mounted on the bodies of the underwater vehicles that can perform real-time water quality surveillance. Arrays of chemical sensors can be batch fabricated on LCP and the entire array can also be surface-mounted as it is on the vehicle. This feature is impractical when it comes to silicon or glass substrate. The process of the device fabrication, dicing, and packaging into flexible arrays with LCP is highly compatible with established MEMS techniques [10]. Particularly, LCP complies with photoresist patterning through lithography, deep reactive ion etching, thin film sputtering and metallization, which allows it to be easily incorporated into the miniaturized chemical sensors for point-of-care applications. In this work, a flexible chemical sensor with sputtered BFE fabricated on an LCP substrate is proposed. Laboratory characterization reveals favorable analytical performance of the sensor. Application for in-situ Zn detection in the seawater is demonstrated by performing a sea trial testing, wherein a flexible sensor array consisting of four LCP chemical sensors is attached to the hull of an autonomous kayak that is remotely operated to perform various missions.

6.2 Fabrication of flexible LCP chemical sensor

The fabrication of the flexible LCP chemical sensor is not complicated and can be accomplished by means of standard MEMS techniques. A schematic describing the steps involved in the sensor fabrication is shown in Figure 6.1. The first step in the fabrication of the flexible LCP chemical sensor was to dip the LCP thin film into Cu etchant to etch away the protective Cu layer (step 1). After thoroughly cleaning with DI water followed by drying with N₂ gas, the LCP thin film was temporally attached to a silicon wafer (4 inches diameter and 300 μ m thickness) using AZ9260 positive photoresist (10 μ m) and then baked on a hotplate at 80 $^{\circ}$ C for 40 min to bond the LCP film to the silicon wafer. A layer of the

photoresist (5 μm) was spin-coated on the LCP thin film and exposed to 365 nm i-line UV light. The pattern of three electrodes was formed after removing the exposed areas of the photoresist in the AZ 400K developer (step 2). Subsequently, a layer of Cr/Au (50/200 nm) was sputtered by using the magnetron sputtering system, in which the Cr layer was used to promote the adhesion (step 3). Thereafter, the entire wafer was immersed in acetone for 12 hrs to remove the unexposed photoresist along with the metal layer left on top of these areas (step 4). Similar processes were performed to pattern and deposit Ag/AgCl (150/250 nm) reference electrode (step 5 and 6) as well as Bi (200 nm) working electrode (step 7 and 8). The counter electrode of the sensor was defined by the previously-sputtered Au layer (step 3) without any further modification. Photograph of an individual sensor after fabrication is shown in Figure 6.2(a). The LCP chemical sensor can be easily flexed without damaging the thin-film electrode layers. Such high flexibility of a fully bent sensor using a tweezer is depicted in Figure 6.2(b). Prior to experiments, the fabricated sensor was packaged by wiring the contact pads of three thin-film electrodes with conductive epoxy (purchased from Epoxy Technology, USA) and baked at 80 $^{\circ}\text{C}$ for 3 hrs. Following this step, a few drops of non-conductive epoxy (purchased from Epoxy Technology, USA) was used to cover the contact pads and the entire sensor was baked at 80 $^{\circ}\text{C}$ for 1.5 hrs to ensure proper insulation.

Figure 6.1: Detailed fabrication procedures of the flexible LCP chemical sensor, where metal layer left on top of the unexposed photoresist is not shown in order to improve clarity.

Figure 6.2: (a) Photograph of a flexible LCP chemical sensor after fabrication, where WE, working electrode; CE, counter electrode; RE, reference electrode. (b) Photograph of a fully bent sensor using a tweezer to show its high flexibility.

6.3 Chemicals, apparatus and measurement procedures

The chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich, Singapore unless stated otherwise. DI water ($18.2 \text{ M}\Omega\cdot\text{cm}$) collected from a Milli-Q system (Millipore, Singapore) was used for the preparation of all the solutions. A sheet of LCP 3908 ($100 \text{ }\mu\text{m}$ thickness) with two-sided Cu ($18 \text{ }\mu\text{m}$ thickness) protective layer was purchased from Rogers Corporation, USA. PDMS was obtained from Dow Corning, Singapore. NaCl with a concentration of 0.6 mol/L was used for all the laboratory-based experiments. This concentration was determined in accordance with the salinity of seawater, which is about 3.5% [11]. Zn standard stock solution (1000 mg/L) was obtained from Merck, Singapore. Solutions for interference study, including KCl, ammonium chloride (NH_4Cl), magnesium chloride (MgCl_2) and calcium chloride (CaCl_2) were made by dissolving an appropriate amount of powder into the DI water. Solutions of copper nitrate ($\text{Cu}(\text{NO}_3)_2$), lead nitrate ($\text{Pb}(\text{NO}_3)_2$) and cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$) used for interference experiment were prepared from the atomic absorption standard stock solution (1000 mg/L).

Electrochemical experiments were performed by using a portable EmStat3 electrochemical workstation purchased from PalmSens BV, Netherlands. The setup of the laboratory-based experiments is shown in Figure 6.3(a) and 6.3(b), where the packaged LCP chemical sensor was fully immersed in the testing solution contained in a glass vial (Alpha Analytical, Singapore), which was covered by a Teflon cap (Alpha Analytical, Singapore) to prevent falling of dust or other particles into the testing solution. The glass vial was clamped on a magnetic plate, through which stirring of the solution was achieved with the aid of a magnetic stirrer bar placed in the solution. The connection wires of the LCP chemical sensor were connected to the portable electrochemical workstation via crocodile clips. A computer installed with PStTrace 4.7 software was used to control the electrochemical workstation with

measurement parameters being specified based on different experimental objectives. During the sea trial testing, a channel multiplexer (PalmSens BV, Netherlands) and a connection terminal equipped with shielded cables (PalmSens BV, Netherlands) were used to connect the sensor array with the portable electrochemical workstation, which was controlled by remote desktop. All experimental apparatus, including glass vial, Teflon cap, and magnetic stirrer bar were thoroughly cleaned by DI water before the experiments. Eppendorf pipettors (Sigma-Aldrich, Singapore) were used to deliver aliquots of the standard solutions.

Figure 6.3: (a) Photograph of experimental setup of the laboratory-based experiments. (b) Photograph of an enlarged view to show the configuration of the flexible LCP chemical sensor.

CV experiments were carried out under quiescent condition by dipping the flexible LCP chemical sensor in the solution of 0.6 mol/L NaCl. Experimental parameters were chosen as an initial potential of -2.0 V, a final potential of -0.5 V, and a scan rate of 0.05 V/s. SWASV experiments were initiated by accumulating Zn ions on the working electrode under a deposition potential of -1.6 V (after optimization) for 180 s (after optimization). After a 5 s equilibration time, voltammograms were recorded from -1.8 to -1.2 V with a frequency of 10 Hz, an amplitude of 0.02 V, and a step potential of 0.005 V. A conditioning potential of -1.0 V was applied to the working electrode for 120 s for the purpose of removing residual metal ions. During deposition and cleaning/conditioning steps, the solution was stirred at a speed of 500 rpm for all the laboratory-based experiments. For the sea trial testing, seawater was constantly replenished into the PDMS channel during both deposition and cleaning steps by controlling the kayak to move in a straight line. The potentials controlled and measured in all the electrochemical experiments were with respect to the thin-film Ag/AgCl reference electrode. Laboratory-based experiments were conducted at room temperature and solutions were used without purging.

6.4 Working range of the sensor

Considering the material of the working electrode was changed to sputtered Bi and the thickness of the reference electrode was reduced in comparison to the biomimetic chemical sensor, it is necessary to investigate the potential window of the newly designed flexible LCP chemical sensor. The potential window was evaluated by conducting CV experiments with three sensors in the solution of 0.6 mol/L NaCl. The cyclic voltammograms recorded are shown in Figure 6.4(a), where the negative limit of the potential window is about -1.7 V with respect to the thin-film Ag/AgCl reference electrode. When the potential becomes lower than -1.7 V, the magnitude of the negative current starts to dramatically increase which is mainly

resulted from the fast evolution of hydrogen bubbles. The positive limit of the potential window for the sensor is around -0.8 V with respect to the thin-film Ag/AgCl reference electrode. A clear positive current can be found when the potential becomes higher than -0.8 V, which is possibly caused by the oxidation of the Bi layer. A preliminary SWASV experiment was firstly conducted in the solution of 10 $\mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. The voltammogram recorded, as shown in Figure 6.4(b), shows a well-defined stripping peak around the potential of -1.64 V, suggesting that the flexible LCP chemical sensor is suitable for Zn detection.

Figure 6.4: (a) Investigation of potential window of three flexible LCP chemical sensors in the solution of 0.6 mol/L NaCl. Initial potential: -2.0 V; final potential: -0.5 V; scan rate: 0.05

V/s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode. (b) The anodic stripping voltammogram recorded in the solution of 10 $\mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. Deposition potential: -1.4 V; deposition time: 120 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

6.5 Influence of deposition potential and deposition time

In view of the fact that accumulation of target metal ions during the deposition step is affected by the deposition potential as well as deposition time, the influence of deposition potential on stripping peak current of the flexible LCP chemical sensor was further evaluated in the solution of 10 $\mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. The potential was changed in the range between -1.2 and -1.9 V with fixed deposition time of 120 s. As shown in Figure 6.5(a), a rapid increase in the current is observed when the deposition potential is varied from -1.2 to -1.6 V. Increase of peak current is probably contributed by the elevated energy provided to the metal ions to accumulate on the working electrode surface. When the deposition potential becomes more negative than -1.6 V, the stripping current begins to decrease which could be due to the generation of hydrogen bubbles that impede metal ions from being deposited on the electrode surface. Such trend was also observed by Pan et al. [12] using tin-bismuth (Sn-Bi) alloy electrode and Chaiyo et al. [13] using Nafion/ionic liquid/graphene composite modified SPE electrode. Hence, an optimum deposition potential of -1.6 V was selected for remaining experiments. By maintaining the deposition potential at -1.6 V, the influence of deposition time on stripping peak current in the same solution was also studied between 60 and 360 s. The stripping current, as shown in Figure 6.5(b), increases almost in a linear manner. This behavior is possible on account of the reason that Zn ions are able to be constantly supplied to the vicinity of the working electrode as the testing solution is stirred during the deposition process. In view of possible saturation of the working electrode surface

with prolonged deposition time, which usually happens in high concentration samples, a deposition time of 180 s was selected as the optimal value for remaining experiments.

Figure 6.5: Effects of (a) deposition potential and (b) deposition time on stripping peak current of the flexible LCP chemical sensor in the solution of $10 \mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. Deposition time: 120 s for (a); deposition potential: -1.6 V for (b). Potentials were measured with respect to the thin-film Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

6.6 Reproducibility and interference

Having the optimized deposition potential along with deposition time, analytical performance of the flexible LCP chemical sensor in terms of reproducibility over multiple sensors was investigated. The responses of six sensors in stripping peak current during three consecutive SWASV runs are illustrated in Figure 6.6. It can be observed that all chemical sensors exhibit comparable stripping currents. The reproducibility among six sensors calculated from the relative standard deviation (RSD) is 1.64%, suggesting that repeatable responses for Zn detection can be expected with a strategically placed array of the flexible LCP chemical sensors. The influence of possible interfering ions on the analytical performance of the flexible LCP chemical sensor was also investigated, as shown in Figure 6.7. Few common ions, including NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} , Cu^{2+} , Pb^{2+} and Cd^{2+} , were selected owing to their wide availability in the natural water samples. During the experiments, the concentration of interfering ions (1 mg/L) was adjusted to be 100 times higher than the Zn ions (10 $\mu\text{g/L}$). We found that there was no significant interference effect for NH_4 , K, Mg and Ca cations on the Zn stripping signal of the sensor. However, Cu, Pb and Cd ions did have interference effects on the Zn stripping current, which is possibly due to the strong competition between these metal ions to form intermetallic compounds deposited on the working electrode surface. The tolerance limits of the sensor towards Cu, Pb, and Cd ions were around 5, 20 and 20 times higher than the concentration of target Zn ions, respectively. This interference phenomenon was also reported by Meng et al. [14] using Bi-film-modified montmorillonite doped carbon paste electrode and Krolicka et al. [15] using Bi film SPE electrode.

Figure 6.6: Stripping peak current measured during three consecutive runs of SWASV experiments for six flexible LCP chemical sensors in the solution of 10 $\mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

Figure 6.7: Effects of possible interfering ions on stripping peak current of the flexible LCP chemical sensor in the solution of 10 $\mu\text{g/L}$ Zn ions with 0.6 mol/L NaCl. The concentration of NH₄, K, Mg, and Ca ions is 100 times higher than that of Zn ions. While the concentration of Cu, Pb, and Cd ions is 5, 20, and 20 times higher than that of Zn ions. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film

Ag/AgCl reference electrode. Data are presented as the mean of three trials and the error bar denotes the standard deviation of three trials.

6.7 Analytical performance of the sensor

Investigation pertaining to the analytical performance of the flexible LCP chemical sensor was conducted by monitoring responses of several SWASV experiments in the solutions of 0.6 M NaCl with elevated concentration of Zn ions. Figure 6.8(a) shows a series of anodic stripping voltammograms recorded, in which undistorted and legible stripping peaks can be distinguished near the potential of -1.64 V and the magnitude of these peaks becomes higher when the concentration of the testing solution is changed from 0.3 to 70 $\mu\text{g/L}$. The calibration plot is shown in Figure 6.8(b), from which a linear correlation ($R^2=0.999$) between the magnitude of stripping peak current and Zn concentration is obtained. These experimental data reveal that the flexible LCP chemical sensor exhibits linear responses toward Zn ions over the concentration range of 0.3 to 70 $\mu\text{g/L}$ and the sensor displays an analytical sensitivity of 92 $\text{nA}/(\mu\text{g/L})$. Figure 6.8(c) depicts the LOD of the flexible LCP chemical sensor, which is recorded about 0.08 ppb. The LOD was determined by slowly increasing the concentration of Zn ions in the testing solution until a clear stripping voltammogram with a signal-to-noise ratio of at least 3 was observed. A comparison regarding the analytical performance between the flexible LCP chemical sensor and other similar sensors with BFE reported in the literature is listed in Table 6.1. Based on the data presented in the table, it is not an overstatement to claim that the proposed flexible LCP chemical sensor has great potential to be used for trace Zn detection.

Figure 6.8: (a) A series of anodic stripping voltammograms recorded for the flexible LCP chemical sensor with increased concentration of Zn ions from 0.3 to 70 $\mu\text{g/L}$. (b) Corresponding calibration plot of the stripping peak current with respect to the concentration

of Zn ions. (c) The LOD of the sensor recorded in the solution of 0.08 ppb Zn ions with 0.6 mol/L NaCl. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

Table 6.1: Comparison of different chemical sensors with BFE for Zn detection

Working electrode	Sensitivity (nA/($\mu\text{g/L}$))	LOD ($\mu\text{g/L}$)	Reference
Ex-situ sputtered Bi film	92	0.08	This work
In-situ electroplated Bi film	NA [#]	0.3	Xu et al. [16]
In-situ electroplated Bi film	NA [#]	0.4	Demetriades et al. [17]
Ex-situ evaporated Bi film	8.9	3.9	Pei et al. [18]
In-situ electroplated Bi film	12.1	8.5	Pei et al. [18]
Ex-situ screen-printed Bi ₂ O ₃	2.6	33	Khairy et al. [19]

#NA stands for ‘not available’.

6.8 In-situ application of the sensor

With the purpose of exploring potential application of the flexible LCP chemical sensor for in-situ Zn detection, a flexible sensor array was fabricated. As shown in Figure 6.9, four sensors denoted as ‘S1’, ‘S2’, ‘S3’ and ‘S4’ respectively, were placed on a flexible PDMS slab with a thickness of 10 mm. Each sensor was covered by a PDMS channel with a volume of 0.18 cm³ in order to filter coarse particles in the seawater while maintaining continuous water flow which stays in contact with the electrode surface during measurement. As depicted in the inset of Figure 6.9, the channel was designed to have a rectangular inlet (4 mm width $\times$ 3 mm height) and a circular outlet (2.5 mm diameter). An enclosed chamber was inlaid near the main channel to protect the soldering points. Three long narrow slots were used to cover the connection wires. Fabrication of the PDMS channel was accomplished by

casting degassed PDMS solution onto a 3D-printed mold followed by a heat treatment in an oven at a constant temperature of 75 °C for 3 hrs.

Figure 6.9: Photograph of the sensor array used in the sea trial testing, where four flexible LCP chemical sensors are denoted as ‘S1’, ‘S2’, ‘S3’ and ‘S4’. The inset shows the enlarged view of one PDMS channel, in which the chamber is used to protect the soldering points and the slots are used to cover the connection wires.

The sensor array was attached to the hull of an autonomous kayak, as shown in Figure 6.10(a). Edges of each PDMS channel were completely glued by Sil-Poxy silicone rubber adhesive to prevent possible peel-off during measurement, as shown in Figure 6.10(b). Wires of the sensor array were separately connected to different channels of the portable electrochemical workstation, which was mounted inside the kayak. A USB cable was used to connect the electrochemical workstation to one of the onboard computers of the kayak, with which the measurement through PSTrace 4.7 software was remotely controlled. The kayak was equipped with another onboard computer, which was used to remotely navigate the movement of the kayak.

After deployment of the kayak into the sea, the kayak was commanded to move in a straight line, during which the deposition step was executed. Then the kayak was set to be in the stationary mode to collect the voltammogram of each sensor. Thereafter, the kayak was directed to the next location, in between a cleaning/conditioning process was provided to clean the surface of the working electrode. The responses of the sensor array were independently recorded at three locations, denoted as ‘L1’, ‘L2’, and ‘L3’, respectively. The map of these locations calculated based on the GPS coordinates is shown in Figure 6.10(c). In consideration of avoiding possibly-biased measurement results due to potential contamination of the electrode surface, the sensor array was tested one time at each location. Besides, the seawater samples at three locations were also collected so that the concentration of different metal ions at each location can be verified through the commercially established method for later comparison.

Figure 6.10: (a) Photograph of the autonomous kayak used during the sea trial testing, where the yellow arrow shows the direction of the kayak movement and the yellow box shows the location of the sensor array. (b) A close-up of the sensor array attached to the hull of the

kayak. (c) Schematic drawing of the three testing locations, which are denoted as 'L1', 'L2', and 'L3'.

Figures 6.11(a), 6.11(b) and 6.11(c) display the anodic stripping voltammogram recorded during the sea trial testing for the sensor 'S1' at location 'L1', 'L2', and 'L3', respectively. The responses for sensor 'S2', 'S3', and 'S4' at three locations are presented in the Appendix C. For all the sensors, distinct stripping peaks are observed in the potential range from -1.62 to -1.65 V, demonstrating a clear trace of Zn ions at three locations. The water samples collected during the sea trial testing were analyzed by means of ICP-MS and the results are listed in Table 6.2. Results of the ICP-MS analysis confirm the presence of Zn metal ions at all the testing locations. It can be noticed that the concentration of Cu ions is about 1.6-3.3 times higher than that of Zn ions and the concentration of Pb ions is slightly higher (0.8-1.6 times) than that of Zn ions. On the other hand, the concentration of Cd ions is much lower (26.6-51.9 times) than that of Zn ions. These ICP-MS data imply that there is no significant interference effect on the Zn stripping current for the sensor array during the sea trial testing. The quantified Zn concentrations are converted to the current values by using the calibration equation (refer to Figure 6.8(b)). Table 6.3 lists the comparison between the converted current (shown as the reference value) and the stripping peak current detected by each sensor at three locations. It is unambiguous that the sensor array exhibits comparable current values with respect to the reference ones, suggesting promising application of the proposed flexible LCP chemical sensor for in-situ Zn determination. However, there are still some limitations for the sensor to achieve real-life, long-term, multi-location monitoring. For instance, if the water is heavily polluted by other heavy metal ions and/or organic matters, the sensor with current design may not function well in consideration of rapid contamination imposed to the working electrode. In addition, the sensor needs to be thoroughly tested to investigate

possible effects on sensor's output when water properties (including salinity, acidity, alkalinity, hardness, etc.) are changed at different locations of interest.

Figure 6.11: Anodic stripping voltammogram recorded during the sea trial testing for the sensor ‘S1’ at location (a) ‘L1’, (b) ‘L2’, and (c) ‘L3’. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

Table 6.2: ICP-MS results of the seawater samples collected during the sea trial testing

Testing location	Zn ($\mu\text{g/L}$)	Cu ($\mu\text{g/L}$)	Pb ($\mu\text{g/L}$)	Cd ($\mu\text{g/L}$)
L1	0.574 ± 0.017	1.120 ± 0.047	0.717 ± 0.001	0.012 ± 0.003
L2	0.883 ± 0.029	1.379 ± 0.028	0.715 ± 0.0002	0.017 ± 0.001
L3	0.453 ± 0.006	1.508 ± 0.033	0.715 ± 0.0001	0.017 ± 0.003

Table 6.3: Comparison between the converted current and the current detected by the sensor array during the sea trial testing

Testing location	Reference value (μA)	S1 (μA)	S2 (μA)	S3 (μA)	S4 (μA)
L1	5.756	5.868	5.873	5.88	5.918
L2	5.784	5.944	5.97	5.998	6.032
L3	5.745	5.778	5.782	5.838	5.844

References

- [1] World Health Organization. (2011). *Guidelines for drinking-water quality* (4th ed.). Geneva, Switzerland: World Health Organization.
- [2] Roohani, N., Hurrell, R., & Kelishadi, R. et al. (2013). Zinc and its importance for human health: an integrative review. *Journal of Research in Medical Sciences*, 18(2), 144-157.
- [3] Adolfo, F. R., do Nascimento, P. C., & Bohrer, D. et al. (2016). Simultaneous determination of cobalt and nickel in vitamin B12 samples using high-resolution continuum source atomic absorption spectrometry. *Talanta*, 147, 241-245.
- [4] Dias Peronico, V. C., & Raposo Jr., J. L. (2016). Ultrasound-assisted extraction for the determination of Cu, Mn, Ca, and Mg in alternative oilseed crops using flame atomic absorption spectrometry. *Food Chemistry*, 196, 1287-1292.
- [5] Zhao, J., Yan, X., & Zhou, T. et al. (2015). Multi-throughput dynamic microwave-assisted leaching coupled with inductively coupled plasma atomic emission spectrometry for heavy metal analysis in soil. *Journal of Analytical Atomic Spectrometry*, 30(9), 1920-1926.
- [6] Rosende, M., Magalhaes, L. M., & Segundo, M. A. et al. (2014). Assessing oral bioaccessibility of trace elements in soils under worst-case scenarios by automated in-line dynamic extraction as a front end to inductively coupled plasma atomic emission spectrometry. *Analytica Chimica Acta*, 842, 1-10.
- [7] Lamsal, R. P., & Beauchemin, D. (2015). Estimation of the bio-accessible fraction of Cr, As, Cd and Pb in locally available bread using on-line continuous leaching method coupled to inductively coupled plasma mass spectrometry. *Analytica Chimica Acta*, 867, 9-17.
- [8] Tai, C. Y., Jiang, S. J., & Sahayam, A. C. (2016). Determination of As, Hg and Pb in herbs using slurry sampling flow injection chemical vapor generation inductively coupled plasma mass spectrometry. *Food Chemistry*, 192, 274-279.

- [9] Wang, J. (2005). Stripping analysis at bismuth electrodes: A review. *Electroanalysis*, 17(15-16), 1341-1346.
- [10] Wang, X., Engel, J., & Liu, C. (2003). Liquid crystal polymer (LCP) for MEMS: processes and applications. *Journal of Micromechanics and Microengineering*, 13(5), 628-633.
- [11] Seawater. (2016). In *Wikipedia, The Free Encyclopedia*. Retrieved from <https://en.wikipedia.org/w/index.php?title=Seawater&oldid=723318131> (2016).
- [12] Pan, D., Zhang, L., & Zhuang, J. et al. (2012). A novel tin-bismuth alloy electrode for anodic stripping voltammetric determination of zinc. *Microchimica Acta*, 177(1-2), 59-66.
- [13] Chaiyo, S., Mehmeti, E., & Zagar, K. et al. (2016). Electrochemical sensors for the simultaneous determination of zinc, cadmium and lead using a Nafion/ionic liquid/graphene composite modified screen-printed carbon electrode. *Analytica Chimica Acta*, 918, 26-34.
- [14] Meng, J., Li, F., & Luo, L. et al. (2014). Determination of zinc in acacia honey by square wave stripping voltammetry with a bismuth-film-modified montmorillonite doped carbon paste electrode. *Monatshefte für Chemie - Chemical Monthly*, 145(1), 161-166.
- [15] Krolicka, A., & Bobrowski, A. (2016). Employing a magnetic field to amplify zinc signal obtained at bismuth film screen-printed electrodes generated using dual bismuth precursor. *Electrochimica Acta*, 187, 224-233.
- [16] Xu, H., Zeng, L., & Huang, D. et al. (2008). A Nafion-coated bismuth film electrode for the determination of heavy metals in vegetable using differential pulse anodic stripping voltammetry: an alternative to mercury-based electrodes. *Food Chemistry*, 109(4), 834-839.
- [17] Demetriades, D., Economou, A., & Voulgaropoulos, A. (2004). A study of pencil-lead bismuth-film electrodes for the determination of trace metals by anodic stripping voltammetry. *Analytica Chimica Acta*, 519(2), 167-172.

- [18] Pei, X., Kang, W., & Yue, W. et al. (2014). Improving reproducibility of lab-on-a-chip sensor with bismuth working electrode for determining Zn in serum by anodic stripping voltammetry. *Journal of the Electrochemical Society*, 161(2), B3160-B3166.
- [19] Khairy, M., Kadara, R. O., & Kampouris, D. K. et al. (2010). Disposable bismuth oxide screen printed electrodes for the sensing of zinc in seawater. *Electroanalysis*, 22(13), 1455-1459.

7

OTHER APPLICATION, CONCLUSIONS AND FUTURE WORK

This chapter firstly presents the development of an MEMS-based aptasensor for norovirus detection. The aptasensor is built by modifying the biomimetic chemical sensor without micropillar electrode array with biological sensing elements, i.e. norovirus-specific aptamers. The detailed fabrication, visual and electrochemical confirmation of aptamer immobilization, and analytical performance towards murine norovirus determination of the sensor are provided. Thereafter, conclusions and major contributions of this project are summarized. The last section of this chapter points out some possible improvements pertaining to this research topic for future work.

7.1 Norovirus detection using MEMS-based aptasensor

7.1.1 Background and motivation

In addition to employing the MEMS chemical sensor for heavy metal detection, an MEMS-based aptasensor is also developed for Norovirus detection, an extended application in the aspect of biological sensing. Noroviruses (NoVs) formerly known as Norwalk-like viruses, a genetically diverse group of the *Caliciviridae* family, are one of the leading pathogens which have created a number of massive outbreaks of acute viral gastroenteritis around the world [1-4]. NoVs are considered to be highly contagious and it is reported that a very low infectious dose, as few as 18 virus particles, is enough to cause sickness [5, 6]. Symptoms such as nausea, vomiting, diarrhea, muscle pain, headache, and fever may appear depending on the level of exposure to the virus [7]. The infection always quickly spreads in the crowd through person-to-person transmission as well as contaminated food and water. NoV genomes are single-stranded, positive-sensed RNA enclosed in a protein capsid [8]. The capsid structure of the virus has icosahedral geometry, as shown in Figure 7.1. This structure leads to a thermal stability, allowing the virus to survive in the temperature up to 55 °C and in the pH range of 3–7 [9, 10]. The high stability of NoVs makes it very different to decontaminate in the environment, which warrants development of real-time, on-site and rapid detection system. Nowadays, the diagnosis of NoVs is performed by the reverse transcription-polymerase chain reaction (RT-PCR) assays. However, conventional RT-PCR is a complicated approach, which requires numerous time-consuming operations such as RNA extraction, RT reaction (cDNA synthesis) and PCR amplification [11], rendering it unsuitable for on-site and rapid detection of NoVs. Recently, the development of electrochemical sensors modified with biological recognition elements for microbial pathogen detection has gained great attention [12]. Among these, aptamers (DNA or RNA molecules), capable of specifically binding to selected viruses, have shown advantages for biosensing applications.

The unique three-dimensional conformational change of the aptamers upon binding to specific analytes enables the aptasensor to detect NoVs with high specificity and sensitivity [13]. In this work, we aim to develop a miniaturized MEMS-based aptasensor for rapid NoV detection. Murine Noroviruses (MNVs) are chosen as the target viruses in consideration of the structural similarity between MNVs and human NoVs along with the safety of experimental personnel. The biomimetic chemical sensor without micropillar electrode array is used as the sensor platform. The working electrode of the sensor is modified by MNV-specific aptamers. The performance of the aptasensor is evaluated from the electrochemical point of view.

Figure 7.1: Schematic representation of capsid structure of the Norovirus [8].

7.1.2 Fabrication of MEMS-based aptasensor

The MEMS-based aptasensor has similar structure compared to the biomimetic chemical sensor without micropillar electrode array, as shown in Figure 7.2(a) and 7.2(b). Herein, a brief description of fabrication procedures is presented and detailed information can be found in section 3.3.1. The sensor has a Si (500 μm) substrate, on which a layer of SiO_2 (1 μm) was deposited by PECVD to provide insulation for upper electrodes. Before patterning with photoresist, the substrate was thoroughly washed with acetone along with isopropanol and rinsed by DI water to remove impurities. After drying with N_2 gas followed by HMDS

treatment, a layer of positive AZ 9260 photoresist (5 μm) was spin-coated on the substrate and baked on a hotplate. Subsequently, the photoresist was exposed to UV light and fully immersed into AZ400K photoresist developer to form the predetermined pattern. A layer of Cr/Au (50/200 nm) was deposited on the patterned photoresist by DC-magnetron sputter to produce adhesion and base electrode layers, in which the middle and outer semi-annuli are the working and counter electrodes, respectively. Another layer of positive AZ 9260 photoresist (5 μm) was then spin-coated and patterned to define the location of the reference electrode (inner semi-annulus). A layer of Ag/AgCl (300 nm) was sputtered to build a three-electrode configuration.

Figure 7.2: Schematic drawing of (a) top and (b) cross-sectional view of the MEMS-based aptasensor.

The contact pads of three electrodes were wired using conductive epoxy (purchased from Epoxy Technology, USA) and the sensor was baked in an oven at 80 $^{\circ}\text{C}$ for 3 hrs. Finally, the non-conductive epoxy (purchased from Epoxy Technology, USA) was used to cover the entire sensor except for the section of three electrodes. Figures 7.3(a) and 7.3(b) depict

photographs of the sensor base after fabrication. Aptamer immobilization on the Au working electrode under the effect of Au-thiol affinity was performed based on a previous study [13] with slight modification. Prior to immobilization, the sensor was covered by photoresist but leaving the working electrode exposed and thoroughly washed with DI water followed by drying with N₂ gas. The aptamers were immobilized by drop casting the solution of 500 nM HPLC-purified MNV-specific aptamers (HS-5'-AG3) in 20 mM Tris-ClO₄ buffer (pH 8.6) and incubating at 4 °C for 5 days. Thereafter, the sensor was further incubated in the solution of 1 mM β-mercaptoethanol (BME) with ethanol buffer for 5 min to minimize the amount of non-specific adsorption.

Figure 7.3: (a) Photograph of the MEMS-based aptasensor after fabrication. (b) Photograph of an enlarged view to show the three-electrode configuration.

7.1.3 Chemicals, apparatus and measurement procedures

The chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich, Singapore unless stated otherwise. DI water (18.2 MΩ·cm) collected from a Milli-Q system (Millipore, Singapore) was used for the solution preparation. The prototype MNV strain, MNV-1 (clone CW1, ATCC PTA-5935), was propagated on RAW 264.7

(ATCC TIB-71) cell line monolayers with Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum. The propagated MNV particles were purified with gel chromatography (illustra MicroSpin S-300 HR Columns, GE Healthcare Life Sciences, Singapore). The MNV titer was determined by plaque assay as previously described [14]. The MNV-specific aptamers (HS-5'-AG3) were purchased from Integrated DNA Technologies, USA. Solutions of 4 mM $K_3[Fe(CN)_6]$ with 400 mM KCl supporting electrolyte and 4 mM $K_3[Fe(CN)_6]$ with 10 μ M Hexaammineruthenium chloride ($[Ru(NH_3)_6]Cl_3$) in 25 mM phosphate buffer (pH 7) were prepared by dissolving an appropriate amount of powder into the DI water and used for CV and SWV experiments, respectively. The ferricyanide/ruthenium hexamine ($[Fe(CN)_6]^{3-}/[Ru(NH_3)_6]^{3+}$) reporter system has been used to characterize electrochemical sensor for biomolecular sensing [15, 16]. Within the system, the $[Ru(NH_3)_6]^{3+}$ ions are cationic electron acceptors, which undergo reduction to become $[Ru(NH_3)_6]^{2+}$ ions in the vicinity of the working electrode. The generated $[Ru(NH_3)_6]^{2+}$ ions will then move into the solution driven by the concentration gradient. The $[Fe(CN)_6]^{3-}$ ions are anionic electron acceptors, which are initially repelled to the solution under the effect of electrostatic force. The $[Fe(CN)_6]^{3-}$ ions further oxidize the $[Ru(NH_3)_6]^{2+}$ ions to regenerate $[Ru(NH_3)_6]^{3+}$ ions, thereby amplifying the electrochemical signal. During SWV measurements, the magnitude of the current output is directly related to the amount of accessible/unbound aptamers present on the working electrode.

CV and SWV experiments were performed using a CHI 760E electrochemical workstation (CH Instruments, USA) linked to a computer, through which the parameters were specified and the measurements were controlled. The experimental setup is shown in Figure 7.4(a) and 7.4(b), in which the packaged MEMS-based aptasensor was immersed in the testing solution contained in a glass vial (Alpha Analytical, Singapore), which was covered by a Teflon cap

(Alpha Analytical, Singapore) to prevent falling of dust or other particles into the testing solution. The connection wires of the sensor were separately connected to the electrochemical workstation through crocodile clips. Prior to each testing, the open-circuit potential of the cell was checked in order to avoid sudden perturbation to the immobilized monolayer due to potential change. CV experiments were recorded with an initial potential of 0.4 V, a final potential of 0.4 V, a switching potential of -0.3 V, and a scan rate of 0.06 V/s. SWV experiments were recorded in the potential range of -0.3 to 0.4 V with a frequency of 10 Hz, an amplitude of 0.005 V, and a step potential of 0.004 V. All electrochemical measurements were conducted at room temperature.

Figure 7.4: (a) Photograph of experimental setup of the electrochemical measurements. (b) Photograph of an enlarged view to show the configuration of the MEMS-based aptasensor.

7.1.4 Visual and electrochemical confirmation of aptamer immobilization

In order to visually confirm aptamer immobilization, fluorescent-labelled AG3 aptamers modified at the 3' position with 6-carboxyfluorescein (HS-5'-AG3-3'-FAM) were immobilized on the Au working electrode following the same procedure described above (refer to section 7.1.2). Subsequently, the electrode was rinsed with PBS to remove the excess aptamers. The aptamer-derived FAM fluorescence was observed under a laser scanning fluorescent confocal microscope. The images captured before and after immobilization of the fluorescent-labelled aptamers are illustrated in Figure 7.5(a) and 7.5(b), respectively. Figure 7.5(c) shows the intensity of fluorescence derived from the surface of the sensor after immobilization, where fluorescent signal is only observed on the Au working electrode surface rather than the SiO₂ substrate surface, indicating that the immobilization procedure employed in this study is able to produce self-assembled aptamer monolayer as expected.

Figure 7.5: Images captured under a laser scanning fluorescent confocal microscope (a) before and (b) after immobilization of the fluorescent-labelled aptamers. (c) The intensity of fluorescence derived from the surface of the sensor after immobilization.

The electrochemical confirmation of aptamer immobilization on the Au working electrode was checked by CV experiments. The voltammograms recorded are shown in Figure 7.6, in which the bare sensor without aptamer immobilization displays large redox currents, implying that sufficient electrode area is available for the electroactive ions to access. However, the sensor with aptamer immobilization reveals significantly reduced redox currents. The decrease of current is highly possible due to the formation of a self-assembled monolayer of immobilized aptamers onto the Au working electrode, which would diminish the accessible electrode area. This result is another evidence to prove the successful aptamer immobilization on the Au electrode surface.

Figure 7.6: Comparison of cyclic voltammograms recorded for the MEMS-based aptasensor without (in black) and with (in red) aptamer immobilization. Initial and final potential: 0.4 V; switching potential: -0.3 V; scan rate: 0.06 V/s. Potentials were measured with respect to the on-chip Ag/AgCl reference electrode.

7.1.5 Analytical performance of the sensor for norovirus detection

The response of the MEMS-based aptasensor upon binding between immobilized aptamers and MNV particles was investigated by performing SWV in the solution of 4 mM $K_3[Fe(CN)_6]$ and 10 μM $[Ru(NH_3)_6]Cl_3$ with 25 mM phosphate buffer. The square wave

voltammograms recorded are shown in Figure 7.7, where a significant decrease in the magnitude of peak current is observed immediately after a 50 plaque-forming units per milliliter (PFU/ml) MNVs being added into the testing solution. It could be caused by the fact that MNVs, upon addition, are quickly captured by the immobilized aptamers, resulting in reduced amount of accessible aptamers. This result manifests promising capability of the MEMS-based aptasensor towards rapid MNV detection.

Figure 7.7: Comparison of square wave voltammograms recorded for the MEMS-based aptasensor without (in black) and with (in red) addition of 50 PFU/ml MNVs.

In order to investigate the ability of the MEMS-based aptasensor for MNV quantification, a series of ten-fold dilutions of MNVs from 10,000 to 10 PFU/ml were prepared and separate SWV measurements were conducted for each titer. The square wave voltammograms recorded are shown in Figure 7.8, in which well-defined peaks are obtained and the magnitude of the peak current gradually drops with the increase of MNV titer. This result demonstrates high sensitivity of the MEMS-based aptasensor against different titers of MNVs, indicating potential application of the proposed aptasensor for on-site and fast MNV determination.

Figure 7.8: A series of square wave voltammograms recorded for the MEMS-based aptasensor with increased titer of MNVs from 10 to 10,000 PFU/ml.

7.2 Conclusions and original contributions

To conclude, in this project, we focused on the development of novel chemical sensors by employing bio-inspiration and bio-mimicking design philosophy and by taking advantage of MEMS fabrication technology, to achieve fast and accurate on-site and/or in-situ heavy metal measurement as well as possible biological detection. The first sensor is a miniaturized chemical sensor, which consists of a compact microfluidic channel and an integrated three-electrode configuration, i.e. a bio-inspired Au working electrode, an on-chip Ag/AgCl reference electrode, and a thin-film Au counter electrode. The idea of mimicking shark's olfactory sensing system enabled an innovative attempt to transform conventional 2D flat working electrode into 3D micropillar working electrode array. The source of the inspiration, key factors considered in the design, and procedures/critical points of the fabrication were presented in detail. The solid-state on-chip reference electrode and the shark-inspired micropillar working electrode array were separately characterized from both material and electrochemical point of view. Under optimized experimental parameters, analytical

sensitivity of 32 nA/($\mu\text{g/L}$), linear range of 1 to 130 $\mu\text{g/L}$, and LOD of 0.2 ppb with a very short deposition time of 30 s towards Pb heavy metal ions were obtained. Without the involvement of excessive external operations (e.g. forced mechanical perturbation/stirring), the proposed biomimetic chemical sensor manifested promising application in terms of uncomplicated, fast, and on-site detection of Pb pollution.

The second sensor is a compact chemical sensor, which consists of a flexible LCP substrate and an integrated three-electrode configuration, i.e. a sputtered Bi working electrode, an on-chip Ag/AgCl reference electrode, and a thin-film Au counter electrode. Use of LCP material offered high mechanical strength and flexibility to the sensor, allowing it to be directly mounted on the surface of underwater vehicles for sensing. Fabrication of the sensor was easily accomplished through standard MEMS techniques. Analytical performance of the sensor pertaining to detection of Zn heavy metal ions was comprehensively optimized based on the laboratory experiments. The sensor displayed favorable sensing capability in terms of analytical sensitivity of 92 nA/($\mu\text{g/L}$), linear range of 0.3 to 70 $\mu\text{g/L}$, reproducibility with RSD of 1.64% ($n=6$), and LOD of 0.08 ppb. Application of the sensor for in-situ Zn measurement was successfully demonstrated by performing sea trial testing at three locations with a flexible sensor array attached to the hull of an autonomous kayak. The proposed flexible LCP chemical sensor showed great potential to be deployed for in-situ real-time determination of Zn contamination.

The third sensor is a disposable MEMS-based aptasensor, which consists of an integrated three-electrode configuration, i.e. an Au working electrode modified by MNV-specific aptamers, an on-chip Ag/AgCl reference electrode, and a thin-film Au counter electrode. Fabrication of the sensor followed the same protocol of the biomimetic chemical sensor

without micropillar electrode array. The aptamer immobilization on the Au working electrode was achieved by the simple process of drop casting. Confirmation of the successful aptamer immobilization was evaluated via both fluorescent and electrochemical approaches. With the aid of SWV experiments, rapid and on-site MNV detection using the proposed MEMS-based aptasensor was preliminarily demonstrated.

In the course of this research, some original contributions have been made to multiple fields, which could lead to the development of next generation of artificial chemical sensors.

- Introduced the bio-inspiration and bio-mimicking philosophy into the design of artificial chemical sensor, which paved a new direction for future research.
- Designed an innovative lab-on-a-chip chemical sensor with 3D working electrode array inspired by the morphological configuration of shark's olfactory sensing system.
- Demonstrated successful fabrication of chemical sensor with 3D fine structures by means of MEMS techniques and evaluated the electrochemical sensing capability of the 3D working electrode array.
- Proposed a new method to construct solid-state thin-film reference electrode which could be directly incorporated into disposable chemical sensors and studied the electrochemical performance of the reference electrode.
- Elaborated the enhanced analytical performance of the shark-inspired chemical sensor with 3D working electrode array from the fluid-structure interaction point of view.
- Employed LCP as a new type of flexible and robust substrate for portable chemical sensors and presented the ease of fabricating LCP-based chemical sensors.
- Demonstrated the feasibility of deploying LCP-based chemical sensors on autonomous underwater vehicles for real-time and in-situ heavy metal detection.

- Designed a compact MEMS-based aptasensor by slightly modifying the proposed biomimetic chemical sensor and exhibited potential application of the aptasensor for biological sensing.

7.3 Future work

This section provides some suggestions and possible improvements that could be implemented to further enhance the sensing performance of artificial chemical sensors for environmental applications. Future endeavors could probably focus on the following aspects.

- As the olfactory sensing systems of pelagic sharks reveal many charming insights into the implementation of fluid dynamics, especially hydrodynamic science, it could be worth to device chemical sensors with different structures of microfluidic channels as well as sensing electrodes to mimic the olfactory sensors of diverse shark species. Such kind of research would allow us to explore more possibilities, which may boost the sensing capability of artificial chemical sensors to a new level.
- Use of computational simulation to investigate the coupling effect between moving fluid and stationary structure on the mass transport of target analytes to the sensing electrodes could provide useful knowledge to optimize the design of artificial chemical sensors. It would be interesting to integrate multiple mass transport modes, i.e. diffusion, convection, and migration, to enhance the sensitivity of artificial chemical sensors.
- As the working electrode plays a critical role in the voltammetric stripping analysis, modification of the working electrode array with novel nanomaterials, such as organic polymers, metal nanoparticles, and 2D or 3D graphene, could substantially improve the performance of artificial chemical sensors to achieve much lower detection limit.
- Surface modification of the working electrode array with biological sensitive elements, such as microorganisms, enzymes, DNA or RNA molecules, analyte receptors, and

antibodies, could expand the application range of artificial chemical sensors towards rapid and in-situ detection of organic pollutants.

- Combination of artificial chemical sensors with other types of miniaturized sensors, including pressure sensor, flow sensor, thermal sensor, as well as different kinds of automated actuators onto the autonomous underwater vehicles, could achieve real-time, multi-function environmental monitoring and remediation.

Herein, we describe the conceptual design of another type of bio-inspired artificial chemical sensor. As illustrated in Figure 7.9(a), this sensor consists of two parts. The bottom part is a glass sensor base on which three kinds of solid-state, on-chip electrodes, i.e. counter, reference, and working electrode, are integrated. The top part is a microfluidic channel through which testing solution with target analytes is dispensed from the top inlet to the side outlet. Above the bottom working electrode, as shown in Figure 7.9(b), there is a circular array of protruding sensing electrodes. A thin layer of hydrogel is formed to cover the entire electrode array. Incorporation of this hydrogel layer also takes the inspiration from shark's olfactory sensing system. According to the biologists, the olfactory sensory epithelium is covered by a layer of fluidic mucosa with cilia of the olfactory receptor cells extending into this layer [17]. The function of the mucosa is to provide a liquid environment for odorant molecules to dissolve and subsequently to be captured by the olfactory receptor cells. In addition, the mucosa plays the role of filtering large particles and adventurous impurities that could possibly damage the cilia of the olfactory receptor cells. As for the artificial chemical sensor, there are some possible merits that could be attained by coating a hydrogel layer to the sensing electrode array. For instance, the contact time between the sensing electrodes and the testing solution would be increased, thereby significantly minimizing the volume of sample solution required for the testing. Furthermore, the sensitivity of the sensor could be

enhanced as fluidic media provided by the hydrogel would magnify the collection efficiency of target analytes by the sensing electrode array. Finally, the selectivity of the sensor could be also improved in view of the high probability that target analytes with certain properties could be purposely concentrated by adjusting the material of the hydrogel.

Figure 7.9: (a) Schematic drawing of a bio-inspired artificial chemical sensor consisting of a top microfluidic channel and a bottom sensor base. (b) An enlarged view of the sensor base to show the configuration.

References

- [1] Lopman, B. A., Adak, G. K., & Reacher, M. H. et al. (2003). Two epidemiologic patterns of *Norovirus* outbreaks: surveillance in England and Wales, 1992-2000. *Emerging Infectious Diseases*, 9(1), 71-77.
- [2] Lau, C. S., Wong, D. A., & Tong, L. K. L. et al. (2004). High rate and changing molecular epidemiology pattern of *Norovirus* infections in sporadic cases and outbreaks of gastroenteritis in Hong Kong. *Journal of Medical Virology*, 73(1), 113-117.
- [3] Kageyama, T., Shinohara, M., & Uchida, K. et al. (2004). Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to *Norovirus* in Japan. *Journal of Clinical Microbiology*, 42(7), 2988-2995.
- [4] Prato, R., Lopalco, P. L., & Chironna, M. et al. (2004). *Norovirus* gastroenteritis general outbreak associated with raw shellfish consumption in South Italy. *BMC Infectious Diseases*, 4, 1-6.
- [5] Teunis, P. F. M., Moe, C. L., & Liu, P. et al. (2008). Norwalk virus: how infectious is it?. *Journal of Medical Virology*, 80(8), 1468-1476.
- [6] Atmar, R. L., Opekun, A. R., & Gilger, M. A. et al. (2008). Norwalk virus shedding after experimental human infection. *Emerging Infectious Diseases*, 14(10), 1553-1557.
- [7] Norovirus. (2016). In *Wikipedia, The Free Encyclopedia*. Retrieved from <https://en.wikipedia.org/wiki/Norovirus>.
- [8] Hutson, A. M., Atmar, R. L., & Estes, M. K. (2004). *Norovirus* disease: changing epidemiology and host susceptibility factors. *Trends in Microbiology*, 12(6), 279-287.
- [9] Ausar, S. F., Foubert, T. R., & Hudson, M. H. et al. (2006). Conformational stability and disassembly of Norwalk virus-like particles - effect of pH and temperature. *Journal of Biological Chemistry*, 281(28), 19478-19488.

- [10] Cuellar, J. L., Meinhoevel, F., & Hoehne, M. et al. (2010). Size and mechanical stability of norovirus capsids depend on pH: a nanoindentation study. *Journal of General Virology*, 91, 2449-2456.
- [11] Butot, S., Zuber, S., & Baert, L. (2014). Sample preparation prior to molecular amplification: complexities and opportunities. *Current Opinion in Virology*, 4, 66-70.
- [12] Labib, M., & Berezovski, M. V. (2014). Electrochemical aptasensors for microbial and viral pathogens. In M. B. Gu, & H. S. Kim (Eds.), *Biosensors based on aptamers and enzymes* (pp. 155-181). New York, NY: Springer.
- [13] Giamberardino, A., Labib, M., & Hassan, E. M. et al. (2013). Ultrasensitive norovirus detection using DNA aptasensor technology. *Plos One*, 8(11), 1-9.
- [14] Wobus, C. E., Karst, S. M., & Thackray, L. B. et al. (2004). Replication of *Norovirus* in cell culture reveals a tropism for dendritic cells and macrophages. *Plos Biology*, 2(12), 2076-2084.
- [15] Escudero-Abarca, B. I., Suh, S. H., & Moore, M. D. et al. (2014). Selection, characterization and application of nucleic acid aptamers for the capture and detection of human norovirus strains. *Plos One*, 9(9), 1-11.
- [16] Gasparac, R., Taft, B. J., & Lapierre-Devlin, M. A. et al. (2004). Ultrasensitive electrocatalytic DNA detection at two- and three-dimensional nanoelectrodes. *Journal of the American Chemical Society*, 126(39), 12270-12271.
- [17] Cox, J. P. L. (2013). Ciliary function in the olfactory organs of sharks and rays. *Fish and Fisheries*, 14(3), 364-390.

APPENDIX A

Resistance of 60 sensors

Figures A1, A2 and A3 display the resistances of the bottom reference, working and counter electrodes of 60 sensors that were fabricated in three different batches. Among all the 60 sensors, most of them have quite close resistance values of the bottom reference, working, and counter electrodes, which are around 23, 16, and 10 Ω , respectively. However, sensors '6', '16', and '20' in three batches and sensor '10' in the third batch seem to have much higher resistance values than other ones. This disparity could be caused by the nonuniformity of the metal layer deposited on the sensors during the sputtering process. Therefore, these sensors are excluded for further experiments.

Figure A1: The resistances of the bottom reference, working and counter electrodes of 20 sensors fabricated in the second batch.

Figure A2: The resistances of the bottom reference, working and counter electrodes of 20 sensors fabricated in the third batch.

Figure A3: The resistances of the bottom reference, working and counter electrodes of 20 sensors fabricated in the fourth batch.

APPENDIX B

Estimation of Au coverage

CV experiments were performed by separately immersing the commercial Au working electrode (3 mm diameter), the biomimetic chemical sensor without micropillar electrode array and the one with micropillar electrode array into the solution of 0.002 mol/L $K_3[Fe(CN)_6]$ with 0.2 mol/L KCl as the supporting electrolyte. Commercial Ag/AgCl (with 3 mol/L NaCl inner electrolyte) reference electrode and commercial Pt counter electrode were used to minimize the possible bias. The parameters used for the CV experiments were chosen as initial and final potential of 0.6 V, switching potential of -0.2 V, and a scan rate of 0.05 V/s. Cyclic voltammograms of four consecutive scans recorded for different working electrodes are shown in Figures B1, B2, B3, and B4. Since the CV experiments were conducted in the still solution without stirring, the convection factor didn't assist in the movement of the electroactive species, which was the $Fe(CN)_6^{3-}$ ions in the experiments. In addition, the migration factor was also negligible as a sufficient quantity of supporting electrolyte, which was the KCl ions in the experiments, was added into the testing solution. Hence, during the CV experiments, the diffusion factor was the only effect involved to transport the $Fe(CN)_6^{3-}$ ions to the working electrode. Under this condition, the peak current of the CV experiments can be derived from the Randles–Sevcik equation:

$$i_p = 0.4463 \frac{n^{3/2} F^{3/2}}{R^{1/2} T^{1/2}} D^{1/2} \nu^{1/2} A C \quad (1)$$

where i_p , peak current (A); n , number of electrons transferred in the redox reaction; F , Faraday's constant (C/mol); R , gas constant (V•C/mol•K); T , temperature (K); D , diffusion coefficient (cm²/s); ν , scan rate (V/s); A , electrode area (cm²); C , bulk concentration (mol/cm³).

For equation (1), $n = 1$; $F = 96485.33 \text{ C/mol}$; $R = 8.31 \text{ V}\cdot\text{C/mol}\cdot\text{K}$; $T = 298.15 \text{ K}$; $\nu = 0.05 \text{ V/s}$; $C = 2 \times 10^{-6} \text{ mol/cm}^3$; D was same for all the experiments. The Au coverage on different working electrodes can be calculated by comparing the ratio of the peak currents.

Figure B1: Cyclic voltammogram of the first scan for (a) commercial Au working electrode (in black), (b) the biomimetic chemical sensor without micropillar electrode array (in blue) and (c) the one with micropillar electrode array (in red). Initial and final potential: 0.6 V; switching potential: -0.2 V; scan rate: 0.05 V/s. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

By substituting the above-mentioned constant values as well as the values of respective peak currents into equation (1),

1.1 Au coverage on the biomimetic chemical sensor without micropillar electrode array (A_{11})

$$A_{11} = \frac{-6.42 \mu A}{-19.77 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 2.30 mm^2$$

2.1 Au coverage on the biomimetic chemical sensor with micropillar electrode array (A_{21})

$$A_{21} = \frac{-8.40 \mu A}{-19.77 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 3.00 mm^2$$

Figure B2: Cyclic voltammogram of the second scan for (a) commercial Au working electrode (in black), (b) the biomimetic chemical sensor without micropillar electrode array (in blue) and (c) the one with micropillar electrode array (in red). Initial and final potential: 0.6 V; switching potential: -0.2 V; scan rate: 0.05 V/s. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

1.2 Au coverage on the biomimetic chemical sensor without micropillar electrode array (A_{12})

$$A_{12} = \frac{-6.46 \mu A}{-19.56 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 2.33 mm^2$$

2.2 Au coverage on the biomimetic chemical sensor with micropillar electrode array (A_{22})

$$A_{22} = \frac{-8.41 \mu A}{-19.56 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 3.04 mm^2$$

Figure B3: Cyclic voltammogram of the third scan for (a) commercial Au working electrode (in black), (b) the biomimetic chemical sensor without micropillar electrode array (in blue) and (c) the one with micropillar electrode array (in red). Initial and final potential: 0.6 V; switching potential: -0.2 V; scan rate: 0.05 V/s. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

1.3 Au coverage on the biomimetic chemical sensor without micropillar electrode array (A_{13})

$$A_{13} = \frac{-6.46 \mu A}{-19.51 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 2.34 mm^2$$

2.3 Au coverage on the biomimetic chemical sensor with micropillar electrode array (A_{23})

$$A_{23} = \frac{-8.43 \mu A}{-19.51 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 3.05 mm^2$$

Figure B4: Cyclic voltammogram of the fourth scan for (a) commercial Au working electrode (in black), (b) the biomimetic chemical sensor without micropillar electrode array (in blue) and (c) the one with micropillar electrode array (in red). Initial and final potential: 0.6 V; switching potential: -0.2 V; scan rate: 0.05 V/s. Potentials were measured with respect to the commercial Ag/AgCl reference electrode.

1.4 Au coverage on the biomimetic chemical sensor without micropillar electrode array (A_{14})

$$A_{14} = \frac{-6.45 \mu A}{-19.52 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 2.34 mm^2$$

2.4 Au coverage on the biomimetic chemical sensor with micropillar electrode array (A_{24})

$$A_{24} = \frac{-8.45 \mu A}{-19.52 \mu A} \times \frac{\pi \times 3^2}{4} mm^2 = 3.06 mm^2$$

The average value of Au coverage on the biomimetic chemical sensor without micropillar electrode array and the one with micropillar electrode array is $2.33 \pm 0.02 mm^2$ (average of A_{11} , A_{12} , A_{13} and A_{14}) and $3.04 \pm 0.03 mm^2$ (average of A_{21} , A_{22} , A_{23} and A_{24}), respectively.

APPENDIX C

Response of the sensor array during sea trial testing

Figures C1, C2, and C3 show the anodic stripping voltammograms recorded during the sea trial testing for the sensor 'S2', 'S3', and 'S4' of the sensor array at location 'L1', 'L2', and 'L3', respectively. From these figures, well-defined stripping peaks located in the potential range from -1.6 to -1.65 V can be clearly observed, which demonstrates a clear trace of Zn ions that are present at the testing locations.

Figure C1: Anodic stripping voltammogram recorded during the sea trial testing for the sensor 'S2' at location (a) 'L1', (b) 'L2', and (c) 'L3'. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

Figure C2: Anodic stripping voltammogram recorded during the sea trial testing for the sensor 'S3' at location (a) 'L1', (b) 'L2', and (c) 'L3'. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

Figure C3: Anodic stripping voltammogram recorded during the sea trial testing for the sensor 'S4' at location (a) 'L1', (b) 'L2', and (c) 'L3'. Deposition potential: -1.6 V; deposition time: 180 s. Potentials were measured with respect to the thin-film Ag/AgCl reference electrode.

LIST OF PUBLICATIONS

Patents:

- [1] **Wang, N.**, Miao, J. M., & Triantafyllou, M. S. (2015). MEMS chemical sensor for heavy metal detection. *US Provisional Patent*, Application No.: 62/188,824.
- [2] **Wang, N.**, Miao, J. M., & Triantafyllou, M. S. (2017). Chemical sensor for heavy metal detection. *PCT Patent*, Publication No.: WO2017/007427.

Book chapter:

- [1] **Wang, N.** (2016). Biological olfaction inspired chemical sensors. In A. G. P. Kottapalli, M. Asadnia, J. M. Miao, & M. S. Triantafyllou (Eds.), *Biomimetic microsensors inspired by marine life* (pp. 23-51). Cham, Switzerland: Springer.

Journal papers:

- [1] **Wang, N.**, Kanhere, E., Miao, J. M., & Triantafyllou, M. S. (2016). Miniaturized chemical sensor with bio-inspired micropillar working electrode array for lead detection. *Sensors and Actuators B: Chemical*, 233, 249-256.
- [2] **Wang, N.**, Kanhere, E., Kottapalli, A. G. P., Miao, J. M., & Triantafyllou, M. S. (2017). Flexible liquid crystal polymer-based electrochemical sensor for in-situ detection of zinc(II) in seawater. *Microchimica Acta*, 184(8), 3007-3015.
- [3] Kanhere, E., **Wang, N.**, Kottapalli, A. G. P., Asadnia, M., Subramaniam, V., Miao, J. M., & Triantafyllou, M. (2016). Crocodile-inspired dome-shaped pressure receptors for passive hydrodynamic sensing. *Bioinspiration & Biomimetics*, 11(5), 1-14.

- [4] Kanhere, E., **Wang, N.**, Kottapalli, A. G. P., Miao, J. M., & Triantafyllou, M. (2017). Hemispherical array of sensors with contractively wrapped polymer petals for flow sensing. *Smart Materials and Structures*, 26(11), 1-13.
- [5] Kitajima, M., **Wang, N.**, Tay, M. Q. X., Miao, J. M., & Whittle, A. J. (2016). Development of a MEMS-based electrochemical aptasensor for norovirus detection. *Micro & Nano Letters*, 11(10), 582-585.

Conference papers:

- [1] **Wang, N.**, Kanhere, E., Triantafyllou, M. S., & Miao, J. M. (2015). Shark-inspired MEMS chemical sensor with epithelium-like micropillar electrode array for lead detection. *18th International Conference on Solid-State Sensors, Actuators and Microsystems (IEEE TRANSDUCERS 2015)*, USA, pp. 1464-1467.
- [2] **Wang, N.**, Kanhere, E., Triantafyllou, M. S., & Miao, J. M. (2015). Copper detection with bio-inspired MEMS-based electrochemical sensor. *19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015)*, Korea, pp. 23-25.
- [3] **Wang, N.**, Kitajima, M., Mani, K., Kanhere, E., Whittle, A. J., Triantafyllou, M. S., & Miao, J. M. (2016). Miniaturized electrochemical sensor modified with aptamers for rapid norovirus detection. *11th Annual International Conference on Nano/Micro Engineered and Molecular Systems (IEEE NEMS 2016)*, Japan, pp. 587-590.
- [4] **Wang, N.**, Kanhere, E., Tao, K., Triantafyllou, M. S., & Miao, J. M. (2016). Lead detection using polymer-based electrochemical sensor modified with bismuth nanoparticles. *8th Asia-Pacific Conference of Transducers and Micro/Nano Technologies (APCOT 2016)*, Japan, oral presentation.
- [5] Kanhere, E., **Wang, N.**, Asadnia, M., Kottapalli, A. G. P., & Miao, J. M. (2015). Crocodile inspired dome pressure sensor for hydrodynamic sensing. *18th International*

Conference on Solid-State Sensors, Actuators and Microsystems (IEEE TRANSDUCERS 2015), USA, 1199-1202.

[6] Hu, L. X., **Wang, N.**, Miao, J. M., & Gruber, G. (2016). Investigation of temperature dependency on the propulsion of disk-like nanoswimmers. *26th Anniversary World Congress on Biosensors (Biosensors 2016)*, Sweden, 48-50.

[7] Tao, K., Lye, S. W., **Wang, N.**, Hu, X., & Miao, J. M. (2015). A sandwich-structured MEMS electret power generator for multi-directional vibration energy harvesting. *18th International Conference on Solid-State Sensors, Actuators and Microsystems (IEEE TRANSDUCERS 2015)*, USA, 51-54.