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Universitat Autònoma  
de Barcelona

**Cost-effective electrochemical heavy metal  
sensors in environmental monitoring and  
healthcare: from *in-situ* monitoring to a  
wearable format**

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**Ph.D. Thesis**

**Ph.D. in Material Science**

**Director:**

**Prof. Arben Merkoçi**

**Department of Chemistry**

**Science Faculty**

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## Declaration

The present work entitled “Low-cost printed electrochemical sensing platforms applied in environmental monitoring and healthcare”, presented by Qiuyue Yang to obtain the degree of doctor by the Universitat Autònoma de Barcelona, was performed at the Nanobioelectronics and Biosensors Group at the Institut Català de Nanociència i Nanotecnologia (ICN2), under the supervision of Prof. Arben Merkoçi, ICREA Professor and Group Leader.

Qiuyue Yang (Author)

Prof. Arben Merkoçi Hyka (Director)

The present Thesis was performed also under the doctoral program studies “Material Science” at Science Faculty, Universitat Autònoma de Barcelona, under the tutorship of Prof. Arben Merkoçi Hyka.

Prof. Arben Merkoçi Hyka (Tutor)

Bellaterra, September 2022

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## Preface

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The research work during this thesis has been summarized as one published book chapter, two published research articles, and one manuscript submitted to an international peer-reviewed scientific journal.

Introduction (section 1.2.3) has resulted in one book chapter:

Yang, Qiuyue, et al. "Signal enhancement strategies." *Wearable Physical, Chemical and Biological Sensors*. Elsevier, 2022. 123-168. Experimental work in Chapters 2-4 have resulted in two published articles and one article in submission:

Yang, Qiuyue, et al. "Development of a heavy metal sensing boat for automatic analysis in natural waters utilizing anodic stripping voltammetry." *ACS Es&t Water* 1.12 (2021): 2470-2476.

Yang, Qiuyue, et al. "Wearable and fully printed microfluidic nanosensor for sweat rate, conductivity, and copper detection with healthcare applications." *Biosensors and Bioelectronics* 202 (2022): 114005.

Yang, Qiuyue, et al. "Metal-free Cysteamine Functionalized Graphene Alleviates Mutual Interferences in Heavy Metals Electrochemical Detection". Submitted to *Green Chemistry*, 2022 (under review).

Moreover, collaborations during my Ph.D. career have resulted in some publications, however non-related to the thesis topic:

Panáček, David, et al. "Graphene Nanobeacons with High-Affinity Pockets for Combined, Selective, and Effective Decontamination and Reagentless Detection of Heavy Metals." *Small* (2022): 2201003.

Vito-Francesco, De, et al. "An innovative autonomous robotic system for on-site detection of heavy metal pollution plumes in surface water." *Environmental monitoring and assessment* 194.2 (2022): 1-19.

Rosati, Giulio, et al. "A plug, print & play inkjet printing and impedance-based biosensing technology operating through a smartphone for clinical diagnostics." *Biosensors and Bioelectronics* 196 (2022): 113737.

Flauzino, José MR, et al. "Label-free and reagentless electrochemical genosensor based on graphene acid for meat adulteration detection." *Biosensors and Bioelectronics* 195 (2022): 113628.

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# List of Abbreviations

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0-D	Zero-Dimension
1-D	One-Dimension
2-D	Two-Dimension
3-D	Three- Dimension
0DNM	Zero-Dimensional Nanomaterial
1DNM	One-Dimensional Nanomaterial
2DNM	Two-Dimensional Nanomaterial
3DNM	Three-Dimensional Nanomaterial
A	Integral Area of Stripping Peak
AAS	Atomic Absorption Spectroscopy
AgNP	Silver Nanoparticle
ANN	Artificial neural network
ASV	Anodic Stripping Voltammetry
AuNP	Gold Nanoparticle
AuNW	Gold Nanowire
BDD	Boron Doped Diamond
BIA	Batch Injection Analysis
BioFET	Field-Effect Transistor-based Biosensor
CAGR	Compound Annual Growth Rate
CE	Counter Electrode
CF	Cystic Fibrosis
CFU	Colony-Forming Unit
CNT	Carbon Nanotube
CT scan	Computed Tomography Scan
CV	Cyclic Voltammetry
DME	Dropping Mercury Electrode
DMP	Dimatix Materials Printer
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPV	Differential Pulse Voltammetry
EDTA	Ethylenediaminetetraacetate
EIS	Electrochemical Impedance Spectroscopy
EPA	Environmental Protection Agency
FET	Field Effect Transistor
FG	Fluorographene
FIA	Flow Injection Analysis
FSS	Fluidic Sensing System
FTIR	Fourier Transform Infrared Spectroscopy
GA	Graphene Acid

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GCE	Glassy Carbon Electrode
GC-MS	Gas Chromatography-Mass Spectrometry
g-C <sub>3</sub> N <sub>4</sub>	Carbon Nitride
GF	Graphite Fluoride
gFET	Graphene Field Effect Transistor
GO	Graphene Oxide
GOx	Glucose Oxidase Enzyme
GPS	Global Positioning System
GSH	Cysteamine Covalently Functionalized Graphene
GSH-SPCE	Cysteamine Covalently Functionalized Graphene Modified Screen-Printed Carbon Electrode
GUI	Graphic User Interface
HA	Humic Acid
HASB	Hard and Soft Acids and Bases
HM	Heavy Metal
HMDE	Hanging Mercury Drop Electrode
HMIs	Heavy Metal Ions
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ISE	Ion Selective Electrode
ISF	Interstitial Fluid
ITO	Indium Tin Oxide
LC-MS	Liquid Chromatography-Mass Spectrometry
LFA	Lateral Flow Assay
LOC	Lab on a Chip
LOD	Limit of Detection
LOQ	Limit of Quantification
MOF	Metal-Organic Framework
MOSFET	Metal-Oxide Semiconductor Field-Effect Transistor
MWCNTs	Multi-walled Carbon Nanotubes
NFC	Near Field Communication
OI	Operator Interface
PAN	Polyacrylonitrile
PANI	Polyaniline
PCB	Printed Circuit Board
PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
PEDOT	Poly (3,4-ethylene dioxythiophene)
PEDOT: PSS	Poly (3,4-ethylene dioxythiophene) polystyrene sulfonate
PEN	Polyethylene naphthalate
PProDOT	Poly (3,4-proplenedioxythiophene)

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PProDOT(MeSH) <sub>2</sub>	Thiol Functionalized Poly (3,4-proplenedioxythiophene)
PET	Polyethylene Terephthalate
PI	Polyimides
PMEHB	Poly (MPC-co-EHMA-co-MBP)
ppb	Part per Billion
ppm	Part per Million
ppt	Part per Trillion
Ppy	Polypyrrole
PS	Polystyrene
PVA	Polyvinyl Alcohol
R2R	Roll to Roll
RE	Reference Electrode
RFID	Radio-Frequency Identification
rGO	Reduced Graphene Oxide
RI	Reverse Ionophoresis
RSD	Relative Standard Deviation
ROS	Reactive Oxygen Species
SIA	Sequential Injection Analysis
SPCE	Screen Printed Carbon Electrode
SWV	Square Wave Voltammetry
SWASV	Square Wave Anodic Stripping Voltammetry
SW-BiNT	Single-walled Bismuth nanotube
SWCNT	Single-walled Carbon Nanotube
TMD	Transition Metal Dichalcogenide
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
VMSF	Vertically-ordered Mesoporous Silica Film
VOC	Volatile Organic Compound
WE	Working Electrode
ZIF	Zeolitic Imidazolate Framework

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## Abstract

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Conventional chemical analysis for environmental monitoring and healthcare typically requires expensive and time-consuming processes. Low-cost and rapid sensing platforms are highly desired to respond a large demand for *in-situ* sensors. An electrochemical sensor can fulfill this need due to the simplicity, high sensitivity and portability. On the other hand, it is also necessary to further enhance the associated sensing setups to make them more affordable and compact. Therefore, this thesis developed the low-cost electrochemical sensing platforms that integrate sampling, sensing and data transmission in a unified system, with the aim of *in-situ* detection of heavy metals for environment monitoring and healthcare.

In Chapter 1, it is revealed that both the market and academia have an urgent need for affordable sensors. In order to satisfy it, three aspects – electroanalysis, printing technology, and nanomaterial – were discussed and shown to be effective in the sensing application for environmental monitoring and healthcare, which would fulfill the blank space between conventional laboratory analysis and *in-situ* analysis. In these two fields, heavy metal ions are valuable analytes to reflect water quality and health condition, hence, the state of art of *in-situ* heavy metal sensors was summarized. In this investigation, mutual interference as an unresolved issue in electrochemical heavy metal sensors was discussed with possible solutions.

Chapter 2 shows a fully autonomous sensing boat for the determination of heavy metal ions in natural water in an electrochemical way. It automatically realized a low-cost and *in-situ* assessment of sources and pathways of water pollutants. As a comparison, the traditional spatial assessment by manual sampling and off-site detection in a laboratory suffers from the high expense, long time and trained personnel. The most critical sensing component in the boat was a fluidic sensing system (FSS) designed based on the working principle of square wave anodic stripping voltammetry (SWASV). The FSS controlled the testing fluid to flow on the surface of a screen-printed carbon electrode (SPCE) that was embedded in a fixed flow cell during deposition, and to stop at quiescent time. Due to the accumulation of heavy metals in the dynamic flow, the LODs of the FSS were obtained at the ppb level, *i.e.*, 1 ppb of  $\text{Pb}^{2+}$ , 6 ppb of  $\text{Cu}^{2+}$ , and 71 ppb of  $\text{Cd}^{2+}$  for simultaneous detection in the laboratory. As soon as equipped with the FSS, the autonomous sensing boat was challenged in a stream (Osor Stream, Girona, Spain), demonstrating its ability to distinguish the highest concentration of  $\text{Pb}^{2+}$  in an effluent of a galena-enriched mine from other normal sites in the stream.

However, in Chapter 2 an issue occurs to the bare SPCE: the sensitivity of SPCE to  $\text{Cd}^{2+}$  was overshadowed by other ions (*i.e.*,  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ ), termed as the mutual interference issue— one of the open issues for heavy metal detection by SWASV. To address this issue, in Chapter 3, a graphene derivative functionalized with thiol moieties, namely cysteamine covalently functionalized graphene (GSH), was applied to modify the working surface of the bare SPCE. After modification, the GSH showed selective enhancement of the sensitivity of SPCE to  $\text{Cd}^{2+}$

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by 6 times, while it didn't influence the sensitivities to other ions. Based on *Pearson's hard and soft acid and base principle*, the grafted thiols on the GSH can be responsible for the selective enhancement. Besides, the covalent functionalization of GSH intriguingly rendered the GSH-modified SPCE to be reusable in continuous measurements, which is difficult for non-covalent functionalized graphene modified SPCEs. Therefore, this approach seemed eco-friendly, cost-effective, and reusable, compared to the common strategy of embedding metal particles (*e.g.*, Hg and Bi) onto the working electrode. Lastly, the GSH-modified SPCE was used to challenge *in-situ* and automatic tests of tap water with the FSS.

Besides being applied in the field of environmental monitoring, heavy metal sensor are used for healthcare as Chapter 4 demonstrated.

Chapter 4 demonstrates a microfluidic sweat sensor fabricated by various printing techniques for low-cost  $\text{Cu}^{2+}$  ions detection in a wearable format. The concentration of  $\text{Cu}^{2+}$  ions present in sweat is correlated to Wilson's disease and liver cirrhosis, and is therefore an interesting biomarker together with sweat conductivity and volume. Nevertheless, in order to detect  $\text{Cu}^{2+}$  ions in sweat with wearable sensors, several issues need to be addressed *e.g.*, sweat sampling condition, sweat rate normalization, continuous monitoring, and the dependence on cleaning-room fabrication techniques. Herein, the fully printed microfluidic sensor was used to determine the concentration of  $\text{Cu}^{2+}$  ions, sweat conductivity and sweat volume within a specific time, *i.e.*, sweat rate in artificial sweat, and showed a LOD of 396 ppb, a linear range up to 2500 ppb, and a sensitivity of 2.3 nA/ppb to  $\text{Cu}^{2+}$ . Besides, active sampling was designed with the purpose of manipulating sweat sampling *via* integrating a reverse iontophoresis (RI) module in a customized circuit, whose function was auxiliary to a potentiostat. In particular, this customized potentiostat was flexible and small with a wireless smartphone-based communication module.

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# Chapter 1

# Chapter 1: Introduction

## 1.1 Introduction of Sensors and General Issues

A sensor is a device that receives stimuli and responds with output signals (Figure 1.1.1a).<sup>1</sup> Depending on different sensing mechanisms, the sensors are divided into physical sensors, chemical sensors, and biosensors that are used in widespread applications.<sup>2</sup>

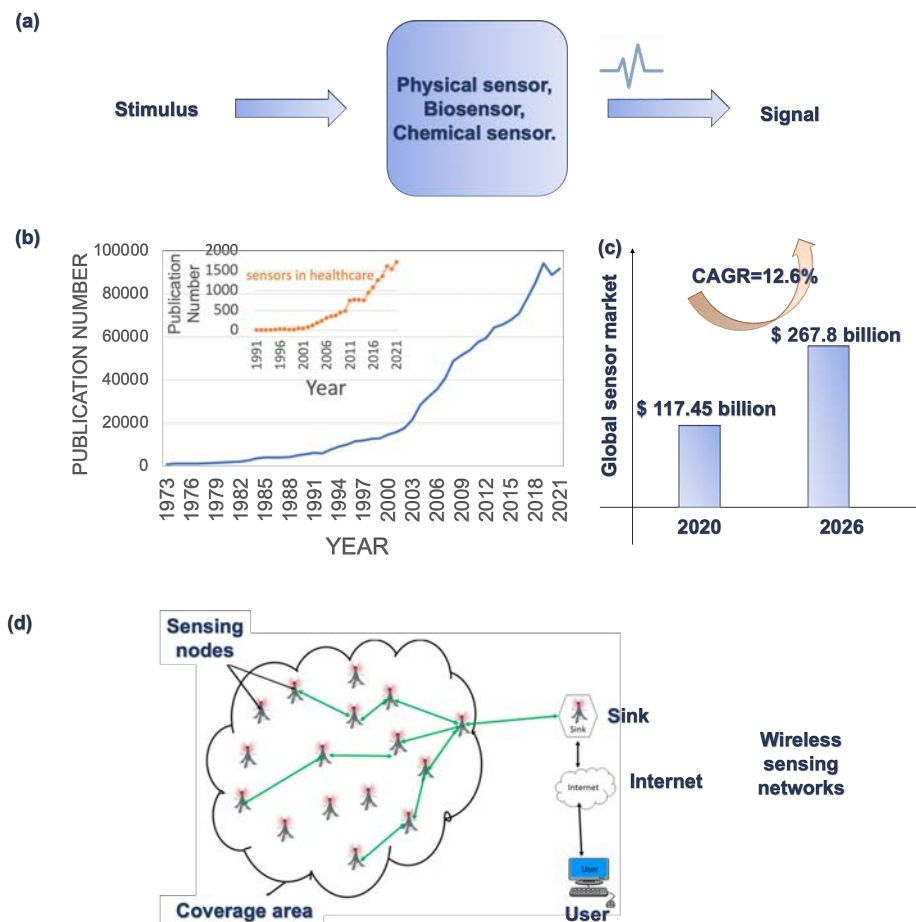


Figure 1.1.1. (a) Schematic illustration of the sensor. (b) The relation of publication number dependence with years using 'sensor' as the keyword in Scopus and inset using wearable sensors in healthcare as the keyword (c) Increased global sensor market in estimation from 2020 to 2026 (d) The wireless sensing networks including numerous sensing nodes.

As the name indicates, the physical sensor can monitor and track physical signals such as temperature, movement, locations, etc. They are easily found in daily life e.g., thermometers, pedometers and Global Positioning Systems (GPS). In the field of healthcare, physical sensors are typically integrated into wearable devices to monitor the physiological signals of human

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beings, such as heartbeat, respiration frequency, pulses, electrocardiography, electromyography, and electroencephalography, *etc.*<sup>3</sup> Referring to the chemical sensor, it is defined (by the International Union of Pure and Applied Chemistry, IUPAC) as “a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal”.<sup>4</sup> The chemical sensor transmits warning signals when detecting the analytes with interest *e.g.*, toxic gases, harmful pesticides and hazardous pollutants, and thus, it has been widely applied in industry, agriculture, and environmental monitoring.<sup>5</sup> Although the chemical sensor has some overlapping applications and targets with the biosensor, the biosensor is typically regarded as an independent category due to its unique use of bio-elements (*e.g.*, DNA strands, aptamers, antibodies, and enzymes) as biorecognition receptors (bioreceptors).<sup>6</sup> The biosensor detects the bio-interaction of these receptors with specific analytes in samples by generating mainly electrical and optical signals *via* a transducer, and then the signals are further analyzed and transmitted by a processor so that people can read them feasibly.<sup>7</sup>

In such miscellaneous applications, sensors have become the most fundamental element for they obtain numerous pieces of information and offer a great amount of data in the era of modern information technology nowadays. As such, the development of sensors and corresponding sensing networks has shown its significance in both academia and the market.

As shown in Figure 1.1.1b, the yearly publication number of “sensor” in Scopus (using “sensor” as the keyword with a total number of 1,343,124) demonstrates an obvious increasing trend with time. These publications primarily focus on the fields of environmental monitoring, agriculture, astronomy and healthcare. In particular, the publication number of the sensors for healthcare grows rapidly in the recent two decades, meaning that the healthcare sensors have aroused great attention in the scientific community. Besides, the sensors applied in the field of environmental monitoring occupy a ratio of 11.6% in the total publications for their important roles in determining the hazards in the water, soil, air, *etc.*

On the other hand, the future of the global sensor market also seems optimistic to be expanded. The global sensor market is expected to reach an estimated \$267.8 billion by 2026 with a CAGR (Compound Annual Growth Rate) of 12.5% from 2020 to 2026.<sup>8</sup> Amongst diverse categories in the sensor market, a wearable sensor is a special type of healthcare sensor, and its market is estimated to be boosted significantly (CAGR=19.15%, 2021-2026) in the application of self-health monitoring.<sup>9</sup>

Additionally, the demand for a wireless sensing network constructed with numerous sensors (sensing nodes) has been accelerated with the development of data analyzing technologies. These technologies (*e.g.*, big data analytics, artificial intelligence) are useful to analyze the numerous data obtained from the sensing networks and make a decision for users (Figure 1.1.1d). With the assistance of these technologies, a fully smart grid integrating automatic sampling, sensing, analyzing, and responding wirelessly completely as the form of the “Internet of Things (IoT)” will be favorable.<sup>10</sup> For this reason, the market of wireless sensor

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networks, whose global market is estimated at \$123.93 billion by 2026 at a CAGR of 17.64% over the forecast period 2021 – 2026, is also expected to expand significantly.<sup>11</sup> The massive demand for wireless sensing networks also facilitates the need for its basic sensing nodes—sensors.

Facing the great demand for sensors, conventional analytical techniques (*e.g.*, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)) are limited to responding although they are highly sensitive and selective. Typically, they have limitations such as operational complexity, high analysis cost, large time consumption, and the requirement for laboratory condition and trained personnel.<sup>12</sup> In the environmental monitoring field, the pollutants in water (such as pesticides, microplastics, antibiotics, steroids, hormones, *etc.*) are frequently determined by high-performance LC-MS,<sup>13–15</sup> GC-MS,<sup>16</sup> and Fourier transform infrared spectroscopy (FTIR).<sup>17</sup> In the field of healthcare, to determine hazardous chemical compounds, viruses, tumor cells, and pathogens in the human body, the analysis implanting in clinics and hospitals in developed regions is typically required. For example, the gold standard method for the diagnosis of SARS-Cov-2 infection still relies on polymerase chain reaction (PCR) amplification and Computed Tomography Scan (CT scan), which requires a complicated extraction and amplification process, and the determination by bulky equipment.<sup>18</sup> Nonetheless, a rapid antigen test for Sars-Cov-2 based on lateral flow assay (LFA) has been widely used as a point-of-care diagnosis at home. Other sensors *e.g.*, glucose sensors, pregnancy test papers, and smartwatches have achieved success in the market, too.

Furthermore, the conventional analysis methods bring one more issue: they are difficult to operate for *in-situ* measurement which requires the integration of sampling, sensing, and analyzing steps in one device. However, the *in-situ* measurement is highly desired because it opens a facile path of real-time monitoring, which can be greatly useful to construct the smart sensing grid. In the case of healthcare sensors, the *in-situ* measurement is very convenient to monitor interesting analytes in real time, assisting in timely intervention for disease. On the other hand, *the in-situ* measurement can keep the most original information of analytes in their sample matrices (*e.g.*, natural water, sweat and saliva) without requiring additional storage and transport.<sup>19</sup>

Hence, the facilely operational and mass-productive sensors for *in-situ* measurement for environmental monitoring and healthcare are urgently needed to cover the blank space where conventional analysis cannot.<sup>20,21</sup> Innovative sensors/sensing platforms must be developed further to realize the high-level integration (of sampling, sensing, and analyzing in one device), the low-cost fabrication and small size.

In the next section 1.2, three aspects, namely electroanalysis, printing techniques, and nanomaterials are considered to address these issues through their advantages such as

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simplicity, low cost, and enhancement in sensing performance (shown in Figure 1.2.1 as table of content).

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## 1.2 How to Tackle: Electroanalysis, Printing Techniques, and Nanomaterials

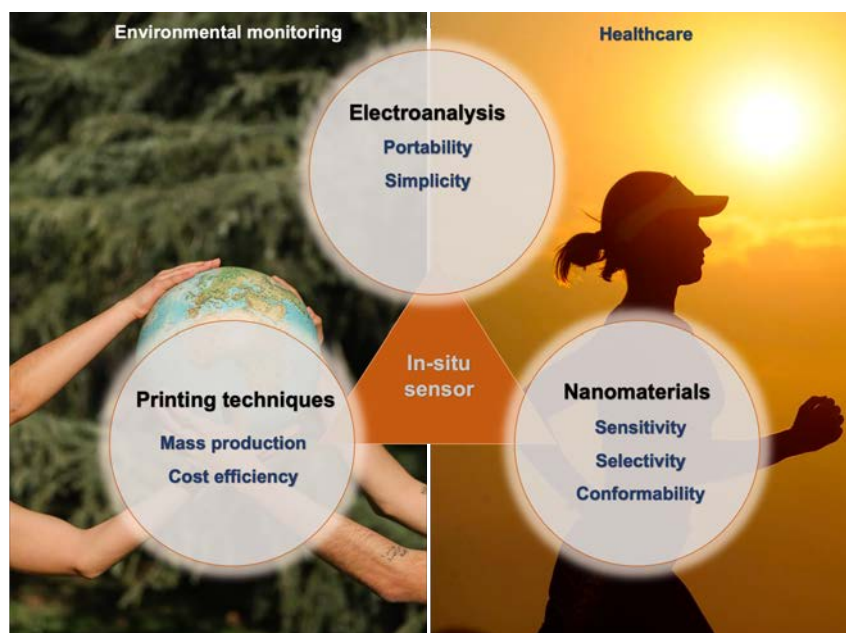


Figure 1.2.1. Table of content of section 1.2: Three aspects, *i.e.*, electroanalysis, printing techniques and nanomaterials, are considered useful in the development of sensors for environmental monitoring and healthcare due to their specific merits.

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## 1.2.1 Electroanalysis in Environmental Monitoring and Healthcare

The evolution of sensors is found to be closely related to the development of electronics and other technologies. The most intuitive impression is the size of sensors that has been significantly decreased, upon the boosted number of transistors in readout devices under Moore's law.<sup>22</sup> Hence, electroanalysis takes advantage of abundant studies and experience during the development, benefiting from the mature fabrication techniques of hardware and corresponding data processing *via* electric signals. Consequently, electroanalysis has been widely applied in the development of miniaturized devices with high portability, and suitable to construct *in-situ* sensing networks in the fields of environmental monitoring and healthcare. Electroanalysis is primarily for electrochemical sensors that will be introduced in the following subsections and some typical sensing platforms are demonstrated, too.<sup>23</sup>

### 1.2.1.1 Electrochemical Sensors

Many cost-effective and *in-situ* sensors in the fields of healthcare and environmental monitoring are developed based on electrochemical detection due to their high sensitivity, simple architecture and digital output signals.

In order to construct an electrochemical cell for sensing application, at least two electrodes and an electrolyte solution are required. One of the two electrodes (working electrode, WE) can respond to the targets, and the other one, termed as the reference electrode (RE), offers the constant potential as a reference. When the sensing system is working in a zero-current mode (potentiometry), the two-electrode system is used as a simple sensing device; however, in the case of the current flowing into the RE, the RE cannot offer a stable potential anymore. Hence, a counter electrode (CE) is added as an independent electrode to build a three-electrode system as shown in Figure 1.2.2a. The quantification of analytes in the three-electrode system is determined *via* the changed electrical signal *e.g.*, current or voltage (Figure 1.2.2b) by monitoring the electrochemical process occurring at the electrode-electrolyte interface (Figure 1.2.2c). Hence, besides potentiometry, other potentiostatic techniques are employed in the three-electrode system, such as cyclic voltammetry (CV), differential-pulse voltammetry (DPV), square-wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS), *etc.* Detailed information has been finely presented in this book,<sup>23</sup> and briefly, a summary about these techniques is given in Figure 1.2.2d.

The conventional electrodes require relatively complex setup and cleaning pretreatment such as a dropping mercury electrode (DME) and hanging mercury drop electrode (HMDE).<sup>26</sup> Nowadays, diverse electrodes with a small size and a simple measuring setup are flourishing in the fields of healthcare and environmental monitoring. Herein, the main types of sensing electrodes applied for on-site measurement are summarized in the following content.

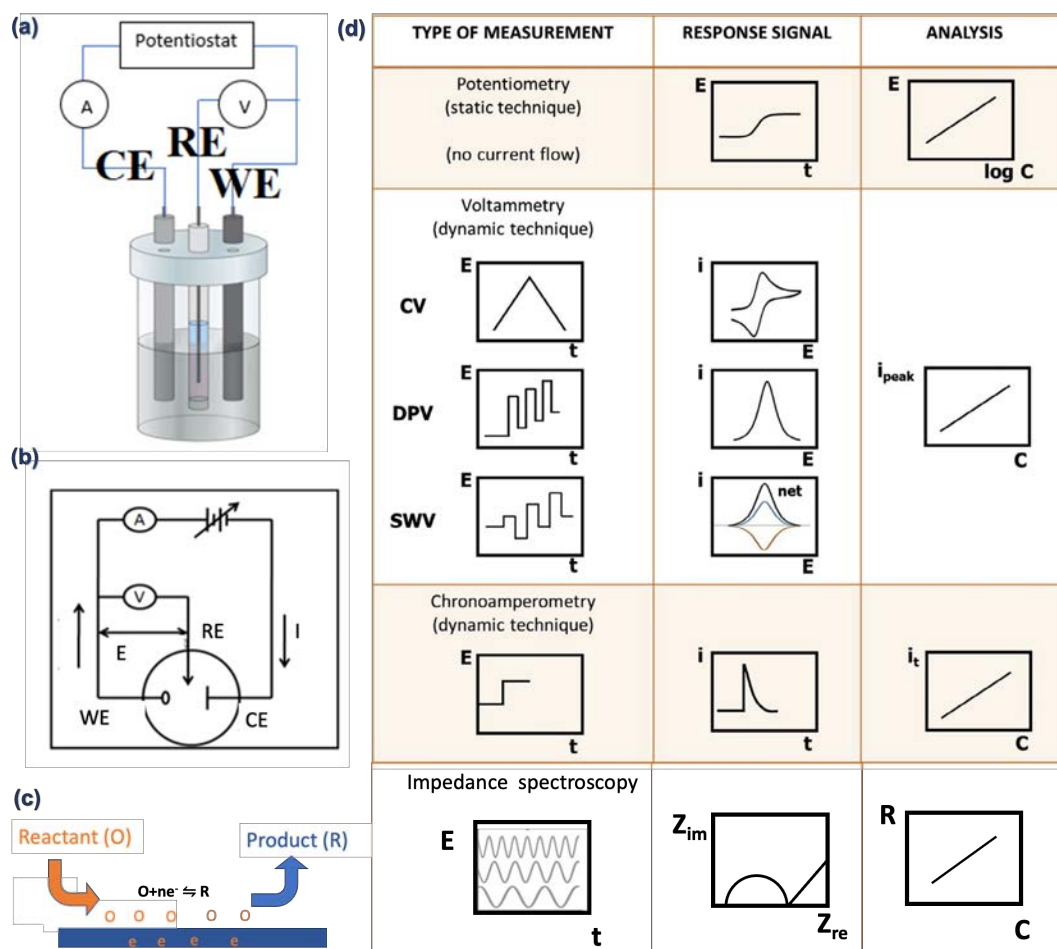


Figure 1.2.2. Scheme of (a) the typical three-electrode system, with the input of applied voltage and the output of the current passing through working and counter electrodes, and (b) the electronic diagram of this system. (c) Working principle of the three-electrode system: the chemical reaction occurring at the interface of electrode-electrolyte at a given voltage initiates the exchange of electrons on electrodes.<sup>24</sup> (d) Summary about commonly used electrochemical techniques in the sensing application.<sup>25</sup>

### 1) Ion-selective electrode (ISE) for ions analysis

Ion-selective electrodes are commonly used in potentiometric techniques to measure the concentration of target ions *via* the voltage across an ion-selective membrane. The membrane only allows some specific ions to enter the internal electrolyte, while it refuses other interfering ions, which results in the change of the voltage between the internal reference electrode and the external reference electrode.<sup>23</sup>

In the field of healthcare, the ISE is typically used to monitor the metabolites in sweat due to its simple and real-time analysis in a wearable format. For example, Zhai et. al. reported a stretchable gold nanowires (AuNWs)-based ion-selective electrode for the *in-situ* measurement of Na<sup>+</sup>, K<sup>+</sup>, and H<sup>+</sup> ions in sweat (Figure 1.2.3a).<sup>27</sup> Modified with polyaniline (PANI), Na ionophore X and valinomycin-based selective membrane (Figure 1.2.3a, inset), the gold-nanowire electrodes can monitor the concentration of H<sup>+</sup>, Na<sup>+</sup> and K<sup>+</sup>, respectively.



These targets are important indicators for skin disease and electrolyte loss. The robustness of this sensor is highlighted due to its stable results even under 30% strain. Besides, a flexible customized circuit including a microcontroller, a battery, and a wireless module, was integrated into the wearable sensing system in order to sample and transmit the data from the sensor to a smartphone wirelessly, ensuring facile operation and high conformability for users.

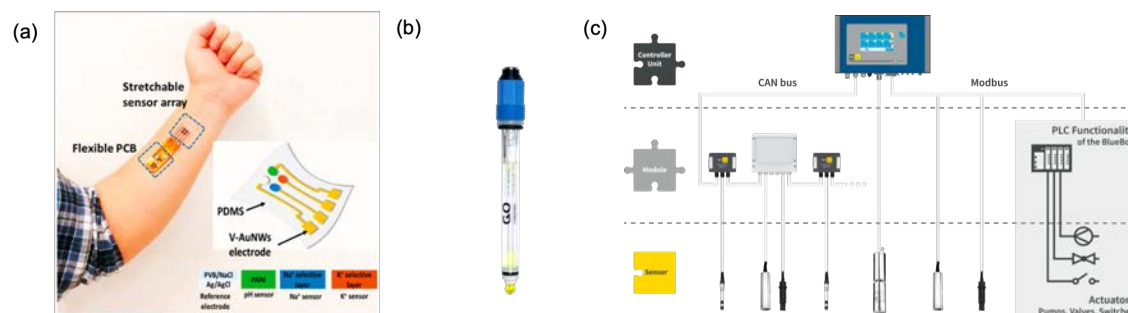


Figure 1.2.3. (a) Photograph of the wearable sensing system for the detection of Na<sup>+</sup>, K<sup>+</sup>, and H<sup>+</sup> ions. (Inset) Scheme of the ISE on a wearable substrate. V-AuNWs is the abbreviation of gold nanowires managed in vertical way<sup>27</sup> (b) Photograph of submersible ISE for detecting pH in natural waters (c) The corresponding commercial readout device controlling several ISEs in parallel.<sup>28</sup>

For environmental monitoring, to date, the ISE has been employed as a submersible sensing probe for water quality monitoring. Some of these probes are commercially available in the market for the on-site measurement of various parameters, *e.g.*, nitrate, ammonium, chloride, and pH (Figure 1.2.3b).<sup>28</sup> The corresponding read-out devices can be also acquired commercially. For example, a sensor manager, called Bluebox (GOSYS®),<sup>29</sup> is able to sample and analyze the data obtained from several ISEs simultaneously, store data in the cloud and communicate with users *via* a tablet or smartphone wirelessly. (Figure 1.2.3c)

Briefly, the ISEs are highlighted for the facile measurement and simplex read-out device with high commercial availability. However, the long-term stability of the membrane and the severe interference from sample matrices are reported to influence practical use.<sup>30</sup>

## 2) Metal-film electrode for ions analysis

Besides the ISEs, metal-film electrodes are widely used for ion analysis in environmental monitoring and healthcare using anodic stripping voltammetry such as DPV and SWV. In these techniques, after a deposition step for the accumulation and reduction of target ions, the potential is released toward the reverse direction and the current is recorded with the varied potential. The acquired voltammogram is correlated to the specie and concentration of the analyte, respectively.

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The metal-film electrodes (*e.g.* Bi, Au, Hg) evolve after people realizing the toxicity of dropping mercury electrodes (DME) and hanging mercury electrodes (HME), demonstrating high sensitivity and simplicity in heavy metal ions (HMIs) detection for water quality control.

Tercier-Waeber et. al showed one example of a submersible sensing probe to monitor multiple HMIs (*i.e.*, Hg<sup>+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, As<sup>3+</sup>) in the aquatic system.<sup>31</sup> The sensors, composed of gold nanofilaments and Hg hemispheres, were embedded in a flow cell. The electronic housing, including a potentiostat and several pre-amplifiers, realized the communication interface for data storage and transition. Due to its miniaturized size, the sensing probe could be equipped on a boat driven into the lake to realize the *in-situ* measurement without any sample pretreatment.

Besides electrolyte ions (*e.g.*, Na<sup>+</sup>, K<sup>+</sup>), certain HMIs (*e.g.* Cu<sup>2+</sup>, Zn<sup>2+</sup>) in sweat are interesting to be monitored due to their involvement in pathological condition. For instance, the disorder of the Cu<sup>2+</sup>/Zn<sup>2+</sup> ratio could correlate with coronary heart disease.<sup>32</sup> Accordingly, the metal electrodes on the epidermal substrate are emerging to detect these ions in sweat.

For instance, Gao et. al. reported a wearable microsensor array, monitoring Zn<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup> and Hg<sup>2+</sup> ions in sweat *via* SWASV.<sup>33</sup> The microsensor array contained four microelectrodes, namely, biocompatible gold and bismuth working electrodes, a silver reference electrode, and a gold counter/auxiliary electrode. To compensate for the effect of skin temperature, this sensor was calibrated with the change of the skin temperature measured by a temperature sensor. Besides the human sweat, a urine sample was also collected for cross-validation through inductively coupled plasma mass spectrometry (ICP-MS). Even though the readout system in this study relied on a standard potentiostat which is not practical to be worn on skin, such a real-time evaluation of heavy metals in sweat has great potential for the early warning of overloaded heavy metal (HM) exposure.

### 3) Enzyme-based electrode

Enzymes are proteins that can catalyze chemical reactions under optimum conditions efficiently and selectively. Enzymes offer recognition and amplification functions to enzyme-based electrochemical sensors which are substantially used for healthcare and environmental monitoring. The immobilized enzymes on the working electrode of the sensor either generate or consume electrons when sensing a detectable species.<sup>23</sup>

In healthcare, metabolites (*e.g.*, glucose, lactate, uric acid, and ascorbic acid) are typical analytes detected by the enzyme-based electrochemical sensor. The concentration of glucose in blood is closely related to chronic hyperglycemia,<sup>34</sup> and hence the development of wearable real-time glucose sensors has been promoted recently. A glucose oxidase enzyme (GOx) oxidizes glucose, accompanied by other chemical reactions *e.g.* the reduction of O<sub>2</sub> or some mediators, which can be detected by sensing electrode as shown 1<sup>st</sup>-3<sup>rd</sup> generation in Figure 1.2.4a.<sup>35</sup> To avoid the direct sampling from the blood, other biological fluids such as

tears, saliva, interstitial fluid (ISF) and sweat could use as the sample due to non-invasive or at least minimally invasive detection, for the concentration of glucose in these biofluids correlates with the one in blood.<sup>36</sup>

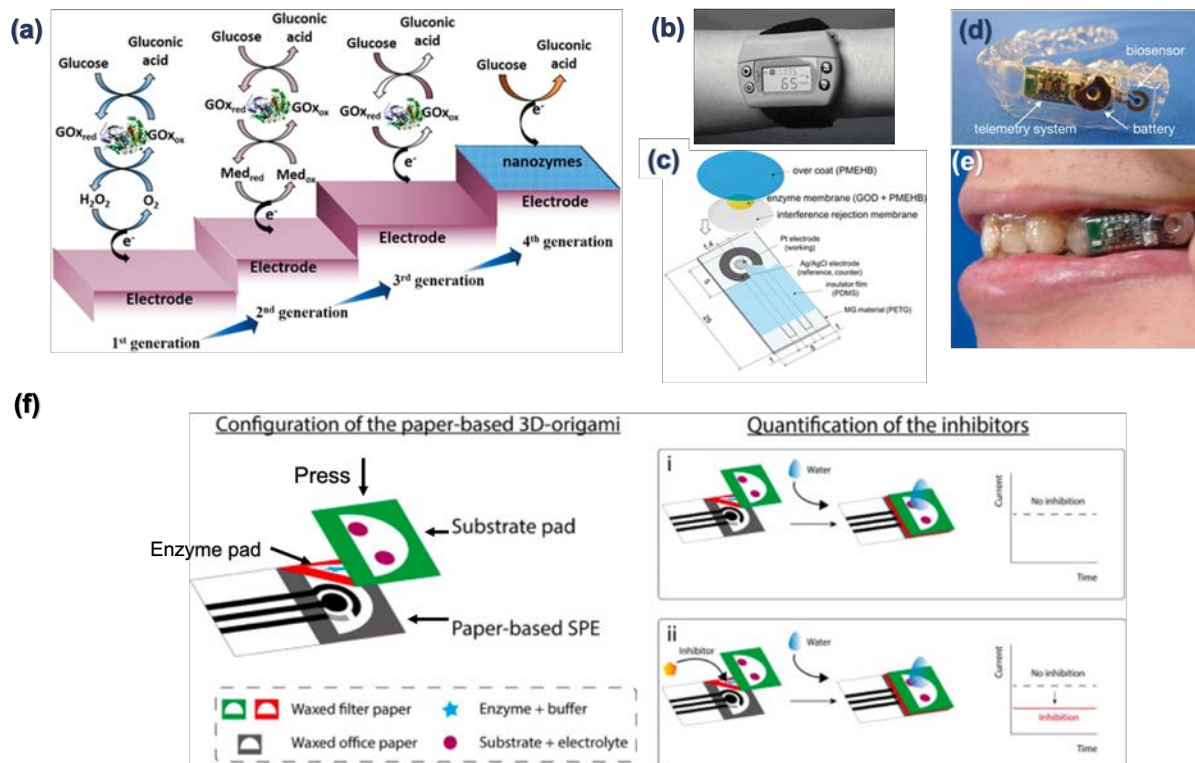


Figure 1.2.4 (a) Schematic illustration of the electrochemical glucose sensor.<sup>35</sup> (b) Photograph of wearable glucose sensor from GlucoWatch®.<sup>37</sup> (c) Architecture of glucose sensor detecting glucose in saliva, (d) photograph of its integration with a mouthguard, and (e) its photograph when being worn by the subject in the mouth.<sup>38</sup> (PDMS is the abbreviation of polydimethylsiloxane.) (f) Details of the pesticides measurement *via* enzymatic activity using carbon electrodes printed on paper.<sup>42</sup>

For example, a commercial glucose sensor called GlucoWatch® was launched by Cygnus Inc., and became the first U.S. Food and Drug Administration -approved glucose sensor (Figure 1.2.4b). The sensor first extracted ISF from skin by reverse iontophoresis (RI), when a mild current forced ions to migrate across the skin *via* two wearable electrodes. Positively charged ions such as Na<sup>+</sup> and K<sup>+</sup> flowed toward the cathode, which generated electro-osmotic flux and drove neutral glucose in the same direction. A GOx-based electrode (which can directly detect intermediate product H<sub>2</sub>O<sub>2</sub>) measured the glucose concentration in the ISF extracted by the RI technique. A user-friendly readout system including data analysis and internal storage was able to send an alarm when the concentration change is greater than 35%.<sup>36,37</sup>

Another example was reported by Arakawa et.al. that a miniaturized sensing device was embedded in a mouthguard, monitoring the glucose concentration in saliva in real time.<sup>38</sup> The GOx-modified poly (MPC-co-EHMA-co-MBP) (PMEHB) membrane was covered on a Pt electrode used as a H<sub>2</sub>O<sub>2</sub> sensor (Figure 1.2.4c). The whole sensor was incorporated into a

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sensing system together with a battery, an analyzing circuit and Bluetooth module for data transmission (Figure 1.2.4d-e). To alleviate the interference from other molecules in saliva, a cellulose acetate membrane was placed on the top of the glucose sensor during validation. The sensing system was able to determine the glucose concentration in the range of 1.75 – 10 000  $\mu\text{mol/L}$ , which fully overlaps the typical salivary sugar concentration of 20–200  $\mu\text{mol/L}$  in mouths.

In the field of environmental monitoring, enzyme-based electrodes are typically used to detect pesticides and HMs, *via* their enzymatic inhibition effect.<sup>39</sup> Pesticides *e.g.*, organochlorine, organophosphate and carbamate pesticides are typical toxic pollutants with a long half-life and able to bioaccumulate in the food chain. The concentration of pesticides has a close correlation with the enzymatic activity, which can be used in enzyme-based electrodes as pesticide sensors.<sup>40,41</sup>

Arduini et al. reported a paper-based and reagent-free electrochemical sensor to detect multiple pesticides (*i.e.*, paraoxon, 2,4-dichlorophenoxyacetic acid and atrazine) *via* their inhibition effect on the enzymes of butyrylcholinesterase, alkaline phosphatase, and tyrosinase, respectively (Figure 1.2.4f).<sup>42</sup> The three different kinds of enzymes (and corresponding substrate) were pre-loaded in multiple filter-paper-based pads. When analyzing diverse pesticide targets in a real sample *via* chronoamperometry, the filter paper-based structure was changed by folding and unfolding, allowing these different enzymes to be released from different pads into the electrode and determine the concentration of these pesticides without additional reagents and sample treatment. In the validation test of real river water, this paper-based device was able to detect paraoxon, 2,4-dichlorophenoxyacetic acid and atrazine at ppb level.

Enzymatic electrodes featuring high sensitivity and selectivity have great potential for *in-situ* detection of environmental pollutants and metabolites in biofluids. Particularly, necessary substrates and enzymes can be pre-stored in papers, realizing direct and simplex measurement. However, enzymatic electrochemical sensors are found to have high cost and stability limitations which may cause the loss of selectivity and susceptibility to enzymatic inhibitors. For this reason, some inorganic nanomaterials have been studied with a trend to substitute for conventional enzymatic electrodes.<sup>43–46</sup>

#### 4) *Bio-affinity-based electrode*

Unlike the previously introduced sensors detecting ions and small molecules, bio-affinity-based electrochemical sensors exploit the selective binding of certain biomolecules (*e.g.*, antibodies, DNA, RNA, and aptamers) toward specific target species to generate electrical signals (Figure 1.2.5a).<sup>47,48</sup> The highly specific binding reactions lead to high selectivity and sensitivity of bio-affinity-based sensors such as Immunosensors,<sup>49</sup> genosensors,<sup>50</sup> aptamer sensors,<sup>51</sup> *etc* in healthcare and environmental monitoring.<sup>52,53</sup>

Amongst them, immunosensor is widely used in the application of healthcare with a typical paradigm of a sandwich structure: antibodies are first modified on the WE as the bioreceptor and then is bound to the tested antigen during measurement; after a specific incubating period for conjugation and necessary washing, a second antibody with an electroactive label attach to the surface of the tested antigen. This label can amplify electrochemical sensing signals, giving rise to sensitive and selective sensing performance. However, this typical paradigm involves several washing steps, and the detection of antigens (*e.g.*, pathogens and proteins) is difficult to operate for *in-situ* measurement which needs direct and simple testing processes.<sup>54–56</sup> Hence, label-free sensing platforms are favorable in this case.

For example, Xu et al. demonstrated a flexible and label-free impedimetric biosensor to detect a biomarker called vascular endothelial growth factor (VEGF) which is the indicator of several diseases including cancers (Figure 1.2.5b).<sup>49</sup> To construct a wearable biosensor to detect VEGF, a composite conductive ink, consisting of photoreactive silk sericin and a conductive polymer, was patterned on a free-standing and conformal substrate—a photo-cross-linkable fibroin thin film (Figure 1.2.5c). Antibodies against VEGF were immobilized on the surface of the patterned WE. When detecting VEGF, the sensor requires incubation time (15 min) to allow the bioreceptors to capture targets. Afterward, the charge transfer resistance on the sensor interface was significantly increased. The change of resistance demonstrated a linear relationship with a wide detection range from  $1 \text{ pg mL}^{-1}$  to  $10 \text{ ng mL}^{-1}$  in both human and artificial urine using a readout device of a commercial bulky potentiostat.

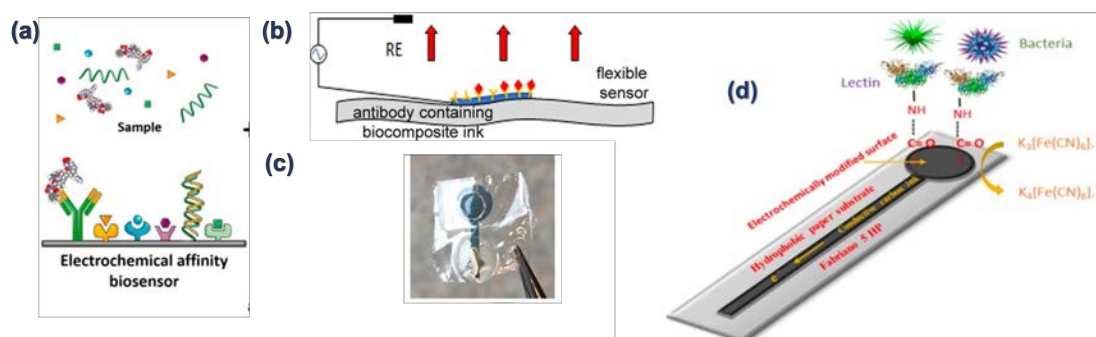


Figure 1.2.5. Scheme of electrochemical affinity biosensor.<sup>48</sup> (b) Label-free impedimetric biosensor to detect VEGF, and (c) its digital image on the flexible substrate.<sup>49</sup> (d) Schematic representation of the functionalized screen-printed probe for bacterial detection.<sup>60</sup>

In environmental monitoring, immunoassay, DNA- and aptamer-based electrochemical sensors have been reported to detect pesticides,<sup>26</sup> HMIIs,<sup>57</sup> and microbial pathogens.<sup>58</sup> However, due to the matrix effect of real samples (*e.g.*, various lakes, rivers and canals), these platforms consume a majority of time to alleviate this effect on complicated sample pretreatment. For instance, the extraction of analytes from real samples requires multiple steps for accumulation and purification. As such, microfluidic devices have been employed to integrate these procedures such as pretreatment, purification and signal amplification into one device.<sup>59</sup>

Paper-based microfluidic devices are emphasized herein for their multiple functions of filtration, fluidic manipulation and pre-storage of necessary reagents which can be easily realized by origami.<sup>58,61</sup> Rengaraj et al. reported a paper-based sensing probe to detect the bacteria extracted from sewage.<sup>60</sup> The probe was fabricated by screen printing carbon ink onto a hydrophobic paper, and then functionalized with carboxyl groups (-COOH). Afterward, a bacteria lectin, called Concanavalin A, was immobilized on the surface of the probe covalently *via* a peptide bond (Figure 1.2.5d). The lectin acted as bioreceptors against the tested bacteria. The sensor was put into trial operation using EIS. A linear sensing range from  $10^3$  to  $10^6$  CFU/mL was obtained with an estimated LOD of  $1.9 \times 10^3$  CFU/mL.

### 1.2.1.2 Bio-Field Effect Transistor (Bio-FET) Sensors

In traditional field-effect transistors (namely metal-oxide-semiconductor field-effect transistor, MOSFET), the current between source and drain ( $I_{ds}$ ) is dictated by gate potential  $V_{gs}$  which tremendously affects the channel conductance of the semiconductor material when drain potential  $V_{ds}$  is fixed. Based on it, field-effect transistors (FETs) are modified by biological elements as receptors (Bio-FETs) and exploited to construct label-free sensors to monitor bioreactions due to their high sensitivity, particularly as *in-situ* and even wearable devices. Bio-FETs can detect the charge distribution of the channel (Figure 1.2.6a) leading to the variation of FET channel conductance when the binding of bioreceptors and target analytes (*e.g.*, ions, nucleic acids and proteins) occurs.<sup>62,63</sup> As such, the surface potential is determined by the concentration of the target analytes, which changes the current between the source and drain (Figure 1.2.6b).<sup>64</sup> Briefly, the Bio-FET sensor consists of two main components: a bioreceptor to recognize target analytes and a FET to detect varied charge distribution caused by the binding of analytes and bioreceptors.

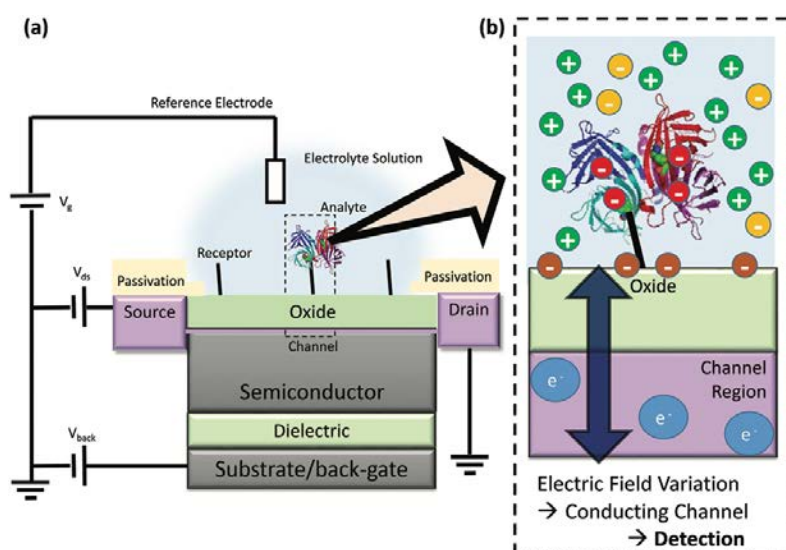


Figure 1.2.6. Schematic diagram of FET operation (a) Example structure of a bioFET sensor, a back-gate within this text refers to an electrode applied to the substrate on which a bias can be applied ( $V_{back}$  can instead simply be grounded). (b) The binding of analytes such as

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streptavidin (shown as ribbons) results in a change in the electric field at the interface and thus a change in the carrier concentration within the conducting channel. This induces a measurable change in current between the source and the drain. The source and drain connections are protected from contact with the analyte solution, and only the gate surface (shown in green) is functionalized for specific binding of the analyte.<sup>62</sup>

Early Bio-FETs generally rely on conventional semiconductive materials (*e.g.* Si) and dielectric metal oxides (*e.g.* Ta<sub>2</sub>O<sub>5</sub>, SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub>). However, the early FETs were often limited in sensitivity due to their low surface-to-bulk ratio. Nanomaterials like carbon nanotubes (CNTs) or graphene with high surface-to-bulk ratios boost the development of Bio-FET sensors with high sensitivity in the applications of healthcare and environmental monitoring.<sup>65,66</sup> Additionally, taking advantage of the flexibility of these nanomaterials, Bio-FET sensors can be used in conformable wearable sensors.

In healthcare, cortisol is monitored as a typical type of stress hormone correlated to individual emotion like depression and anxiety. An et al. presented a wearable aptasensor to detect cortisol concentration in sweat in real time. A semiconductor layer was deposited by electrospinning conducting polyacrylonitrile (PAN) nanofibers, followed by the vapor deposition of carboxylated poly (3,4-ethylene dioxythiophene) (PEDOT) on a silk substrate with Au electrode as the source and drain. The cortisol aptamers as bioreceptors were conjugated on the surface of PEDOT-PAN nanofibers. A liquid-ion gated FET on the silk substrate was then transferred to the polyethylene terephthalate (PET) substrate to achieve better robustness. Lastly, this sensor showed a LOD of 10 pM only within 5s. In real-time validation, it can detect cortisol of up to 1 nM in sweat.<sup>67</sup>

For environmental monitoring, Liu et al. reported a Ti<sub>3</sub>C<sub>2</sub>T<sub>x</sub>-based FET sensor to detect Ag<sup>+</sup> within a few seconds with easy operation and good sensitivity. The layered Ti<sub>3</sub>C<sub>2</sub>T<sub>x</sub> material was deposited onto the interdigital electrodes (*i.e.*, source and drain) as the channel material. The fabricated Ti<sub>3</sub>C<sub>2</sub>T<sub>x</sub>-based-FET sensors demonstrated a LOD of 0.615 μM toward Ag<sup>+</sup> ions without requiring any additional probes or labels. The conductance of the channel formed inside Ti<sub>3</sub>C<sub>2</sub>T<sub>x</sub> is governed by an *in-situ* chemical reaction of Ag<sup>+</sup> reduction to Ag nanoparticles (AgNPs) which is induced by Ti<sub>3</sub>C<sub>2</sub>T<sub>x</sub>. Finally, the fabricated sensors were challenged in real samples of tap and river waters. Good accuracy was observed in tap water; however, there was an unexpected decrease in the sensitivity in river water, which can be related to the water matrix effect in practical use. Generally, the attempt of qualitative Ag<sup>+</sup> sensing application in real water samples is instructive for fast and on-site chemical sensing.<sup>68</sup>

### 1.2.1.3 Chem-Conductometric Gas Sensors

Semiconductive nanomaterials *e.g.*, Mxenes,<sup>69</sup> some graphene derivatives,<sup>70</sup> and transition metal oxides<sup>71</sup> whose resistance can be changed when detecting certain gaseous analytes. This feature is commonly exploited in a chem-resistive gas sensor. The chemical reaction of target gases with adsorbed oxygen species at the interface of gas and solid results in electrons'

exchange as shown in Figure 1.2.7a, and thus, the resistance of these materials is changed.<sup>72,73</sup> Vice versa, when the gas is desorbed from the material's surface, the resistance is recovered.

The chem-resistive gas sensor seems a good candidate for *in-situ* and real-time measurement of hazardous gases in the environment. Some gases such as H<sub>2</sub>S, and NO<sub>2</sub> in the environment is harmful to health, and cause serious effect on the environment such as acid rain, too.<sup>74,75</sup> Chem-resistive gas sensors have high sensitivity provided by nanomaterials, simplicity of readout system due to its conductimetric throughout signal and reusability. However, the selectivity of chem-resistive gas sensors is not as good as other sensors modified with receptors, always showing responses to a broad range of analytes. Fortunately, tailoring nanomaterials is shown to enhance the selectivity of specific analytes.<sup>76</sup>

Li et al. exhibited a yarn-like sensor embedded in woven textiles to determine environmental NO<sub>2</sub> concentration, which can be worn on clothes.<sup>77</sup> The device was made of reduced graphene oxide (rGO)/mesoporous ZnO nanosheet hybrid fibers, featuring robustness to bending, stretching, and twisting activities. The nanocomposites of rGO/mesoporous ZnO nanosheet hybrid fibers acting as sensing layers were deposited on a fiber substrate. When detecting NO<sub>2</sub> at an increasing concentration, the sensor demonstrated a LOD of 43.5 ppb, with great mechanical deformation tolerance of 3000 bending cycles and 1000 twisting cycles with 65% strain strength. In the comparison of responses toward different gas species, namely, 15 ppm NO<sub>2</sub>, 25 ppm SO<sub>2</sub>, 25 ppm HCHO, 50 ppm NH<sub>3</sub>, and 100 ppm CO, the sensor demonstrated the best response to NO<sub>2</sub> than other vapors with higher concentrations (Figure 1.2.7c-d).

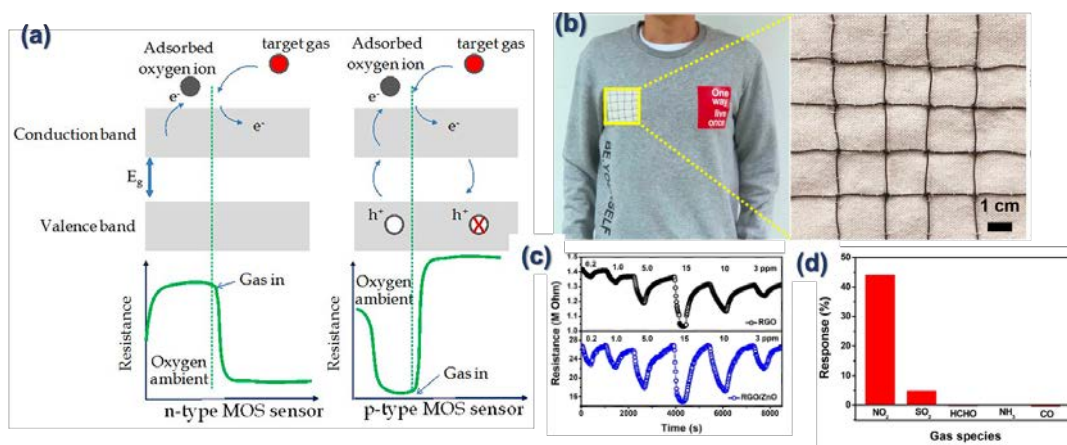


Figure 1.2.7 Schematic diagram of a sensors based on the change of the resistance upon exposure to the target gas (reducing gas) in the cases of n-type and p-type MOS (metal oxide semiconductor) sensors.<sup>73</sup> (b) Digital photograph of the wearable sensor on clothes to detect NO<sub>2</sub> in the environment. (c) Resistance dependence with time when sensing different concentrations of NO<sub>2</sub>. (d) Selectivity diagram showing the response to NO<sub>2</sub> and the responses to other gases at even higher concentrations.<sup>77</sup>

Besides, some gaseous analytes in breath can be monitored for the diagnosis of diseases in an early stage in a non-invasive way. There are two primary approaches: (1) untargeted



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strategy is to acquire breath analysis patterns of healthy people and people with diseases but without requiring the detection of breath biomarkers; (2) targeted strategy is to monitor the breath biomarkers correlated with specific disease using highly selective gas sensors or orthogonal sensor arrays.<sup>78</sup> For example, volatile organic compounds (VOCs) in breath (e.g., alcohols, aldehydes, acids and ketones), are generated by different biochemical and physiological processes. For example, acetone in breath, the biomarker of diabetes, is frequently exhaled more by diabetes patients compared to by healthy people. Similarly, highly concentrated ammonia in exhaled breath is an indicator of kidney malfunction.<sup>79</sup>

Since the outbreak of the worldwide COVID-19 pandemic, chem-resistive gas sensors have been explored to detect the infection of Sars-Cov-2 with the merits of simplicity and rapid detection. For example, Shan et al. reported a sensing array composed of diverse ligands modified AuNPs that either swelled or shrank upon exposure to different VOCs.<sup>80</sup> The volume change of these nanomaterials results in the change in their electric resistance. Hence, a chem-resistive sensor array was constructed, targeting diverse species of VOCs *via* various modified ligands. As such, breath analysis patterns were acquired from healthy people, patients infected by Cov-Sars-2, and other patients suffering from other types of lung diseases in a clinical study in Wuhan, China. A machine-learning algorithm was trained to distinguish the acquired patterns from these three groups. Good discrimination was achieved between the healthy and infectious groups. The obtained data showed 94% and 76% accuracy in differentiating patients from healthy people respectively, as well as 90% and 95% accuracy in differentiating patients infected by Cov-Sars-2 from other lung infections.

#### 1.2.1.4 Electrical Physical Sensors

Unlike those sensors detecting chemical or biological targets, electrical physical sensors can transform events such as light, magnetic field, temperature and mechanical movements into the change of the electrical signals namely, current, voltage, capacitance, resistance and impedance.<sup>81</sup> They are widely used in monitoring ambient parameters in the environment, such as temperature, UV-light exposure and turbidity in the water body.<sup>82</sup>

One example of an ultraviolet (UV)-light sensor has been presented.<sup>83</sup> A photodiode in this sensor generated a photocurrent when exposed to UV-light. The change of current was transmitted to the controlling circuit containing a microcontroller to process data. Then, the obtained data was transmitted to a smartphone *via* an antenna in the sensing system by a near-field communication (NFC) technique which is responsible for both data transmission and power supply from the smartphone. Due to impressively high portability, the sensing platform was miniaturized to be worn on the nails facilely.

On the other hand, for healthcare, numerous electrical physical sensors have been employed in wearable devices to monitor body temperature, physiological parameters, and mechanical movement of human beings.<sup>81</sup>

One paradigm of this kind of wearable sensor is constructed with a dielectric layer embedded in two conductive electrodes on a flexible substrate. When being imposed by bending, pressing, or touching, the deformation of the dielectric layer induces changes in capacitor distance or area shown in Figure 1.2.8a-c, and thus varies the capacitance. Another paradigm is constructed by a conductive layer measured by two electrodes in Figure 1.2.7d,e. When pushing, or stretching, the deformation of the conductive layer results in the change of conductance under Ohm's law. Copious works of literature have piled on the sensors of pulses, voices, and gestures based on this paradigm.<sup>81</sup> In both paradigms, the resistance or capacitance signals then can be processed by a microcontroller, miniaturized multimeter, smartphones, tablets, etc.

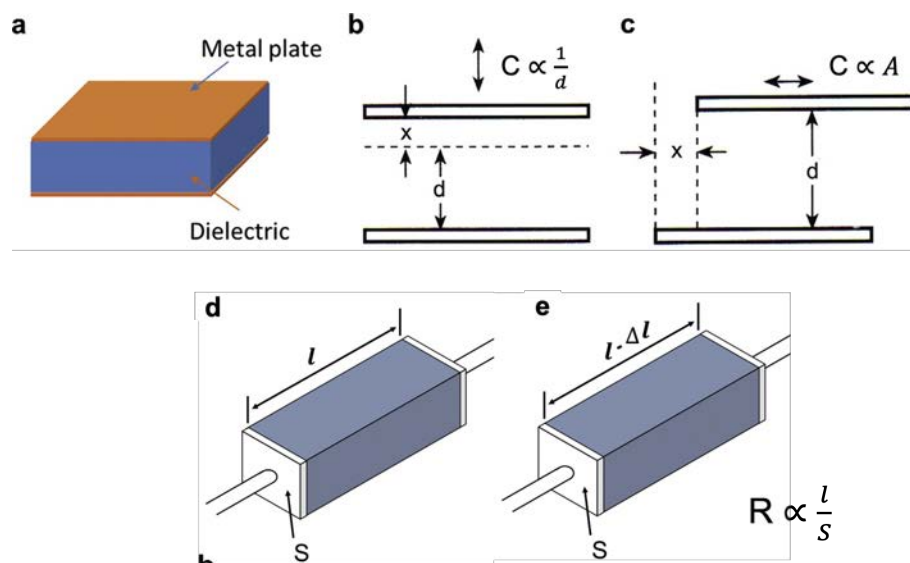


Figure 1.2.8 Schematic illustration of (a) working principle of wearable physical sensors: setup and materials of a capacitive sensor. (b) A capacitive sensor when being pressed by a vertical movement. (c) A capacitive sensor when being stretched by a lateral movement. (d) A conductometric sensor constructed with one conductive layer embedded by two electrodes in (d)original and (e) stretched condition.<sup>81</sup>

Moreover, self-powered sensors are beneficial, for they generate electrical signals without the need for any power supply based on piezoelectric<sup>84</sup> or triboelectric<sup>85</sup> platforms. These sensors can collect the energy of mechanical motion from the human body to power itself, which is advantageous to monitor of specific physiological signals in the long term. For instance, a smart face mask has been reported to detect breath information *via* a self-powered piezoelectric pressure sensor along with a compact readout circuit powered by a lightweight battery. The piezoelectric sensor achieved a peak open-circuit voltage as high as 10 V. The entire sensing system was embedded on the mask due to the small size and light weightness. With the assistance of a wireless communication technique (WIFI module), it transmitted data to smartphones. Finally, the sensing mask was able to distinguish the different breath condition of multiple subjects (Figure 1.2.9).<sup>86</sup>

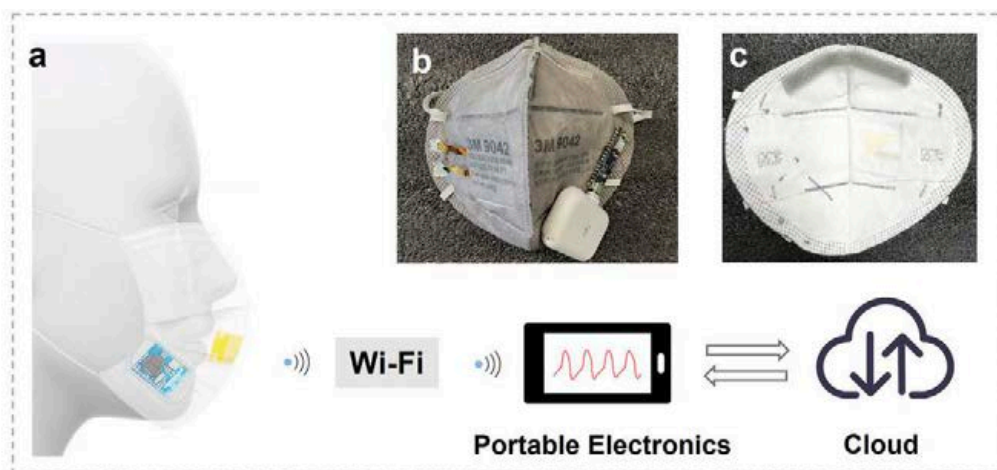


Figure 1.2.9. Design and application of a portable and self-powered pressure sensor. a) Schematic of a smart face mask for wireless breath monitoring. Breath signals detected by the smart face mask were transmitted to portable electronics and updated to the cloud. b,c) Photos of the front (b) and back (c) sides of a smart face mask.<sup>86</sup>

### 1.2.1.5 Summary

In the subchapter of 1.2.1, we summarized the most typical sensing platforms based on electroanalysis for *in-situ* measurement in healthcare and environmental monitoring. The presented sensing platforms share the common properties, *i.e.*, simplicity, portability and high sensitivity. Particularly, electrochemical sensors can detect a wide range of analytes that are valuable for environmental monitoring and healthcare, such as various biomarkers, pathogens and hazardous chemicals.

Aiming for *in-situ* analysis with simple sample pretreatment, lab-on-a-chip (LOC) based sensors have been developed to realize multiple functions in a unified device. This type of devices can allow for fully automatic measurements in an unmanned way in the future. With the development of nanotechnology, the associated power supply and wireless communication modules can be further simplified with higher portability.

Besides simplicity, low cost and mass production can facilitate the proliferation of sensors, which can be addressed by printing technologies. Thus, with this aim, several commonly used printing techniques are introduced in the next subsection.

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## 1.2.2 Printing Techniques for the Fabrication of Electrochemical Sensors

### 1.2.2.1 Printed Electronics

#### 1) Brief introduction

Printing, one of the greatest inventions, has revolutionized culture and history. Before the invention of printing (approximately in the 1400s), the transmission of perspectives to the public was extremely difficult. Nowadays, due to the reduction of printing cost, the dissemination of knowledge in this area has unprecedented acceleration with enormous number of publications such as novels, magazines, newspapers *etc.*<sup>85</sup>

In the area of science and technology, printing techniques also play vital roles in the manufacture of electronics in a cost-effective way, which is considered to fulfill the blank space of microfabrication. Microfabrication is a collection of techniques used to fabricate electronic devices (*e.g.*, MOSFETs and solar cells) in the micrometer range (typically from 0.02 to 100  $\mu\text{m}$ ).<sup>87</sup> It allows for the integration of all these devices on a silicon wafer. Generally, microfabrication consists of several necessary operations: a. High-temperature process, to prepare and oxidize the silicon wafer/substrate with doping process. b. Thin-film deposition on substrates (*e.g.*, to construct metal interconnections). c. Patterning process of the deposited/oxidized film.<sup>87</sup>

High-temperature processes and thin-film deposition are crucial, for influencing the quality of the oxidized/deposited films which highly correlate to the final performance of these devices like diodes and FETs, although the patterning process is also important for determining the real dimensions of devices (*e.g.* the width of the channel in FET which influences its switching performance).

Amongst the techniques in microfabrication, photolithography, a typical method for the patterning process, takes advantage of light with a short wavelength, *e.g.*, UV light to increase resolution in nanometer range based on Abbe's law, in which the resolution is propositional to the light wavelength.<sup>88</sup> In this method, photoresist polymer is first deposited as a film (*e.g.*, negative photoresist polymer), and then, with the cover of a photomask, the exposed polymer is crosslinked while the unexposed part is dissolved by other solvents. In this way, specific area of the substrate or pre-deposited thin films is exposed and can be further etched to form the pattern (Figure 1.2.10). Consequently, the resolution of photolithography can be below 20 nm with extreme ultraviolet.

However, for typical microfabrication techniques are employed in either high-volume or high-value manufacture, the cost could hurdle sensor spreading in resource-limited regions. The high cost of ownership does not only originate from the equipment but also from the extremely clean fabrication condition. These microfabrication techniques are operated in a cleanroom, requiring the continuous circulation of purified airflow and minimal vibration of temperature and humidity. Besides, professional personnel instead of untrained people are

necessary to work in cleanrooms, while dressing in specific gowns to prevent particle emissions and minimize disturbance.<sup>87</sup>

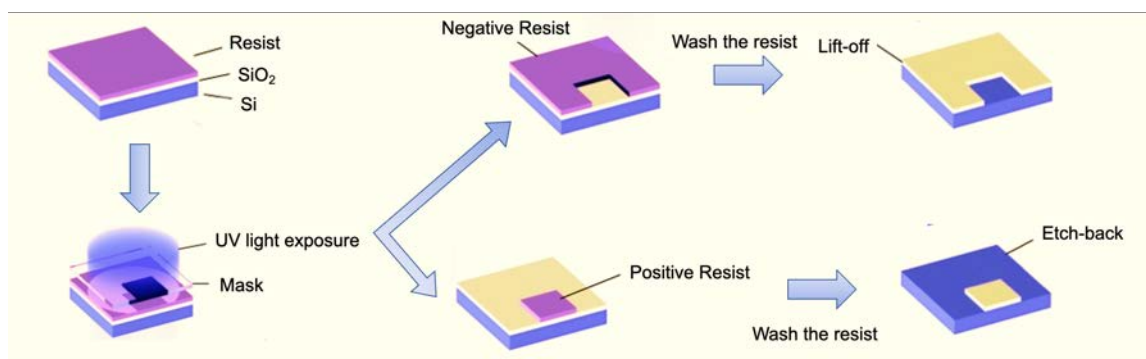


Figure 1.2.10 Schematic presentation of photolithography using negative photoresist which is crosslinked and positive photoresist which is resolved after UV-light exposure respectively.

Unlike microfabrication, printed techniques have some merits such as relatively simple fabrication, limited material wastage and low fabrication cost. It is noteworthy that the printed techniques are typically operated in normal condition in laboratories and could be operated in offices or even at home, opening an easy path to the future scenario of fabricating low-cost electronics in daily life. Due to its low ownership cost, the printing technique as a promising candidate for microfabrication could facilitate the spread of cost-effective sensors, which can be analogous to what printing techniques have done in the dissemination of knowledge in culture and history.

## 2) Milestones in the history of printed electronics

To date back the history of printed electronics, Albert Hanson, is believed to be the first one to introduce the concept of printed electronics. In 1903, a patent for “Printed Wires” filled by him sparked the field of printed electronics.<sup>88</sup> In this patent, the copper foil was proposed to form a pattern on a printed circuit board (PCB) *via* cutting and stamping on a dielectric substrate. In 1936, the first printed circuit was fabricated by Paul Eisler with the feature of large-scale production. Then, in 1948, the printed circuit as a commercial technology was launched in the US. With the discovery of conductive and semiconductive organic materials in the 1980s, organic electronics such as organic FETs, organic photovoltaics, and organic light-emitting diodes initiated to arouse a lot of interest in the scientific community.<sup>89</sup> Accordingly, the printable organic materials promoted the development of printed electronics on a flexible substrate. In 1994, Francis Garnier’s research group firstly reported printed organic FETs on plastic substrates. Although only the electrodes were printed and the organic semiconductors were deposited by vacuum evaporation, it demonstrated the possibility of transistors on plastic substrates, opening the era of flexible electronics. The first fully screen-printed transistors were reported by Zhenan Bao in 1997, showing all printed functional layers including conductor, semiconductor, and dielectrics on a PET substrate.<sup>90</sup> On the other hand, the low charge mobility and long-term stability are the hurdles for organic electronics over three decades of its development history. Hence, with the exploration of

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nanomaterials, diverse inorganic nanomaterials (*e.g.* nanoparticles, nanowires, and nanotubes) have been printed as nano inks to fabricate inorganic printed electronics. In 1999, Jacobson et.al, reported a fully printed inorganic thin-film transistor using cadmium selenide nanocrystals on a silicon wafer, firstly demonstrating the printable inorganic nanomaterials without requiring high-temperature annealing.<sup>91</sup> Nowadays, the composites of organic and inorganic nanomaterials are used to increase their carrier mobility, which helps printed electronics to evolve from the basic PCBs to a wide range of applications such as radio-frequency identification (RFID), photovoltaic, electroluminescent, sensing technologies, *etc.*

### 3) *Typical inks and materials in printing techniques*

Depending on different applications, certain inks containing different functional materials can be selected to deposit and pattern on the substrate. There are three typical types of inks/materials: conducting, semiconducting and dielectric materials.

Regarding conductive materials, the most common ones are metallic nanomaterials such as AgNPs and Au nanoclusters. Unlike bulky metals, metallic nanomaterials have increased surface energy and reactivity, allowed to be sintered at a temperature considerably below the melting points of the corresponding bulky metals. This feature renders metallic nanomaterials to be printed on substrates that cannot sustain high-temperature annealing (*e.g.*, papers).<sup>89</sup> Besides, the high carrier mobility of the metallic conductive ink is higher than organic conductive polymer; hence, they are frequently used to design the conductive paths of circuits and high-Q antennas in RFID technology. On the other hand, some of them are suffering from the high cost due to scarcity (*e.g.*, Au and Ag); some metallic pastes have spontaneous oxidation even at room temperature (*e.g.*, Cu and Al).

To compensate for these limitations, organic conductive polymers have been exploited. Controlling the level of doping by either n-type or p-type dopants, the resultant work function of the conductive polymer can be tuned close to that of the inorganic semiconductors. There are several commonly used conductive polymers such as polyacetylene, PANI, polythiophene, and PEDOT: PSS. The doped polyacetylene film with aligned crystalline orientation can even reach a conductivity of  $10^5 \text{ S cm}^{-1}$  which is almost comparable to the one of Cu. However, these printed polymers are found to have poor long-term stability and robustness.<sup>92</sup>

A third strategy using nanocomposites has been favorably employed to avoid the limits of metallic and polymeric materials. In the nanocomposites, a collection of metallic nanoparticles as nano-fillers are inserted in the polymer matrix with a specific ratio. Unlike common conductive polymers relying on the structure with loosely held electrons in their backbones, in a composite the polymer material itself is not required to be conductive; instead, it can serve as an adhesive for other conductive nanomaterials. The conductivity is realized by the blended conductive nanomaterials, such as carbon black, metal powder, *etc.*<sup>93</sup>

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Moreover, semiconductive ink is critical for the performance of printed electronics. Metal oxides are a unique range of semiconductive materials with excellent electron-transport properties. Particularly, transition semiconductor oxides (*e.g.* ZnO and InGaZnO) are promising to fabricate printable thin-film transistors and flat-panel displays. Besides, silicon-based and carbon-based materials (*e.g.* CNTs) are also favorable. However, similar to the inorganic conductive inks, these inks also require a high annealing temperature and difficult formation, and frequently have impurities of surfactants. These issues should be curbed for practical use. On the other hand, organic semiconducting polymers can form colloidal solutions for printing in different solvents without requiring the high-temperature process. The colloidal solution as the ink can be customized for suitable solubility and film-forming ability, which is useful to fabricate flexible FET devices. Several polymers (*e.g.* regioregular poly(3-hexylthiophene) and poly(3, 3'-dialkyltetrathiophene) based printed transistors can reach the electronic mobility of  $0.1 \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$  and the on-off ratio of  $10^6$ , which is approaching to that of amorphous silicon-based devices.<sup>94–96</sup> Similar to the drawbacks in organic conductive materials, the organic semiconductors are also sensitive to the air, and thus, their long stability could be influenced.

In addition to printable conductive and semiconductive materials, dielectric materials are commonly used in printing electronics to construct insulating layers. An insulating layer is often printed with a thickness ranging from several hundred nanometers to several micrometers to stop any electrical leakage. In contrast, the insulating oxidation layer on standard Si-based transistors is only 10 nm thick. To reduce the printed layer thickness but maintain the high capacitance of the insulating layer ( $C_{ox}$ ) required by transistors, using high-permittivity ( $k$ ) materials as inks is an effective way because the capacitance is directly proportional to the dielectric permittivity and inversely proportional to the film thickness.<sup>97</sup> Hence, some inorganic compounds with high permittivity *e.g.*,  $\text{ZrO}_2$  have been used as insulating inks. However, the printed ceramic film frequently has quality problems such as pinholes and cracks, causing deadly short circuits in devices.<sup>98</sup> For this reason, the organic materials (typically with low permittivity) appear more advantageous than the inorganic ones in this regard due to their capability of well-dispersing in solutions and forming uniform films. The composites of high- $k$  inorganic nanoparticles and printable polymers are also emerging to balance the thickness and quality of the printed insulating layer.<sup>97</sup>

#### 4) Ink preparation

Ink preparation can determine the printing quality, adhesion to the substrate and pattern resolution *via* its rheological parameters *e.g.* surface tension and viscosity. Generally, printable ink consists of active materials, binders, additives and solvents. The active material in ink is the most important component to be deposited on the final patterns and realize the conducting, semiconducting, and insulating functions. Binders, as the name indicates, are responsible for binding active materials to each other and to the substrate. However, post-process annealing is frequently necessary to remove the binders. Examples of typical binders

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are polyvinyl alcohol (PVA), polyvinyl pyrrolidone and resins. Additives, *e.g.*, surfactants, defoamers and stabilizers, are employed to tailor the ink properties. For example, the activated carbon can fill the tiny voids generated by large graphene sheets and improve the conductivity. Solvents are responsible for containing other dispersed elements and ensuring certain rheological properties. The solvent ranges from water to organic liquids and its selection is considerably determined by the applied printing technique, with other consideration for the compatibility with ink components and with the substrate as well as the end-use application.<sup>92</sup>

## 5) Summary

Printing techniques can realize mass production with the efficient cost of electronic devices, which can solve the ongoing issues of *in-situ* electrochemical analysis and respond to the massive demand for low-cost sensing nodes. Particularly, the use of microelectrodes in a highly integrated sensing platform appears to become the tendency of electrochemical sensors in healthcare and environmental monitoring in section 1.2.1; however, microfabrication typically relies on the techniques in the cleanroom with high cost. Hence, several printing techniques in standard laboratory condition have been found to fabricate low-cost electrochemical electrodes.<sup>99</sup> Commonly used printing techniques (*e.g.*, screen printing, inkjet printing, wax printing and other printing techniques) in the electrochemical sensor fabrication are summarized below.

### 1.2.2.2 Screen Printing

A screen-printing process consists of several key elements: screens, squeegees, inks/pastes and substrates.

A screen is a polyester film with open and blocked meshes to form a specific pattern, tightly stretched and mounted on a frame (Figure 1.2.11a). During printing, a squeegee moves and squeezes a thixotropic paste on the screen with a given pressure and velocity. The applied paste has a high viscosity without shear force; however, when pressed by the squeegee on the screen, the texture of the paste becomes thin and sheer, which facilitates the paste to penetrate through the open meshes. Since the rest of meshes are blocked by the screen, and thus, the paste can be deposited on the beneath substrate and form a specific pattern with a uniform thickness.<sup>100</sup> Upon dropping on the substrate, the viscosity of the ink turns to be the same as before printing instantly (Figure 1.2.11b). Hence, the viscosity must be optimized, so as to be compatible with mesh dimension, applied pressure and printing speed.

Due to the use of these highly viscous inks, screen printing can print thick films of thickness varying from decades to several hundred micrometers with a single pass, which is challenging for other printing techniques.<sup>101</sup> For the same reason, the cross-section of screen-printed traces tends to be rectangular, which is easier to build stacked multiple-layered devices than



other printing techniques using the inks with low viscosity and much solvent to generate a cross-section of semicircular arc and coffee rings.<sup>93</sup>

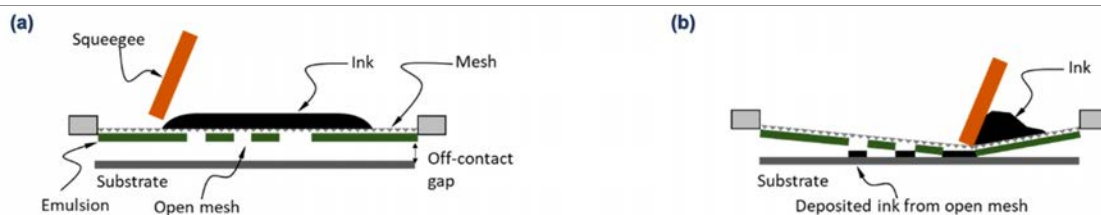


Figure 1.2.11 Working principle of screen-printing technique.<sup>99</sup>

Screen-printing has another feature that is compatible with diverse inks including conductive, semiconductive and dielectric pastes. More excitingly, many pastes are commercially available. For example, Sunchemical Ltd offers carbon/graphite inks, metallic inks (such as Cu, Au, Pt and Ag inks) and dielectric inks.<sup>102</sup> Generally, screen-printed electrochemical sensors can be fabricated using commercially available products in the market. Besides, laboratory-made inks such as carbon, Ag, and Ag/AgCl inks have been reported to fabricate flexible and customized sensors.<sup>103–106</sup> To print a precise pattern, customized inks also require relatively high viscosity, consisting of higher binder loadings compared to the inks by other printing techniques. Ideally, its viscosity ranges from 1000 to 10 000 cps for thin films (of 25–100  $\mu\text{m}$  in thickness), and it can be up to 50 000 cps for a thicker layer (>300  $\mu\text{m}$ ).<sup>93</sup> Generally, the resolution of the screen-printed trace resides in the range of 50–150  $\mu\text{m}$ .<sup>107</sup>

Besides various inks, screen printing is also compatible with a wide range of substrates, such as paper, plastic, ceramic, glass, and metal. While choosing substrates, the curing temperature of specific inks should not cause substrate shrinking to avoid any printed pattern deformation.

For these reasons, screen printing becomes one of the most mature and common techniques to fabricate electrochemical sensors. For example, screen-printed carbon electrodes (SPCE) have been widely used to monitor hazardous pollutants in the environment such as HMs,<sup>108</sup> pathogenic bacteria,<sup>109</sup> and viruses.<sup>110</sup> The commercial inks and relatively simple fabrication render the fabrication to be accessible, low-cost and mass-produced.

### 1.2.2.3 Inkjet Printing

In contrast to screen printing, Inkjet-printing is a drop-on-demand technique that means template-free printing. The pattern is designed through professional graphic software (*e.g.* computer-aided design, CAD) and sent to the printer directly.

The desired two-dimensional shape is formed by the inkjet printer *via* depositing ink droplets row by row. The ink droplet is ejected through nozzles by either a piezoelectric pressure transducer or a thermal bubble. For example, in the piezoelectric mode, a voltage is imposed on the piezoelectric pressure transducer that is placed in the printing head to induce its

physical deformation (Figure 1.2.12a-b). As such, when the transducer bends, an ink droplet is squeezed out of the nozzle (Figure 1.2.12c). With the shape of the transducer recovers, the ink in the channel flows inside the cartridge and gets ready for the next pressure (Figure 1.2.12d). By a periodic voltage waveform, the ink droplet can be ejected continuously.<sup>111</sup>

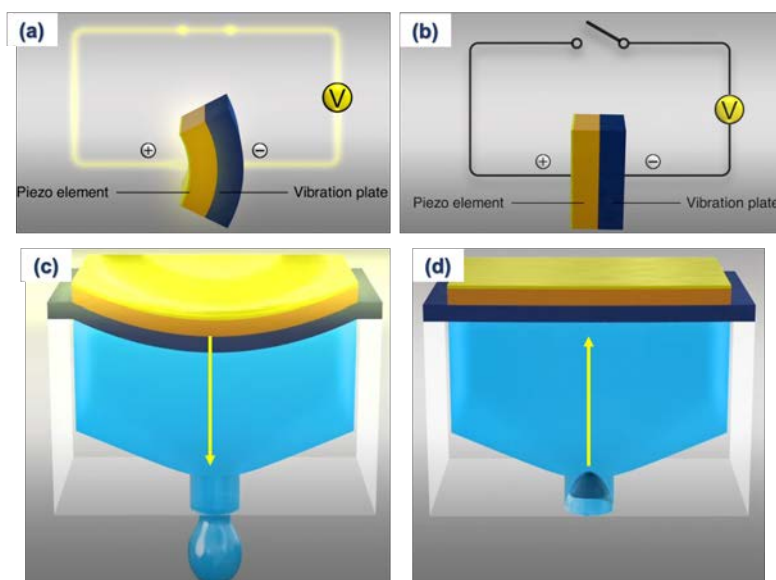


Figure 1.2.12 Piezoelectric actuator (a) bends with imposed voltage and (b) recovers without imposing voltage. Periodic movement of piezoelectric actuator induces (c) ink droplet to squeeze out of the nozzle when actuator bending and (d) be inhaled back into the cartridge when actuator's shape recovers.<sup>111</sup>

Similarly, in thermal-bubble mode, the bubble acts like the piezoelectric transducer to generate necessary deformation. It can expand or shrink under different temperature that is controlled by a tunable current passing through a heating resistor. As such, the bubble causes a periodic pressure dependence with time to propel a droplet of ink out of the nozzle and be ready for the next propelling in a periodic behavior.

Although a multitude of ink materials have proven to be printable, only some of these materials are common to fabricate electrochemical sensors by inkjet printing. For example, conductive materials are frequently used as conductive paths, but semiconductive and insulating materials are rarely used in manufacturing electrochemical sensors by inkjet printing.<sup>112</sup> Most frequently printed conductive materials are metallic nanomaterials such as Au nanoparticles (AuNPs), AgNPs and Ag nanowires, for their size allows them to be propelled through the opening of nozzles (in width of micrometers) to prevent nozzle clogging.<sup>113</sup> Besides, carbon-based nanomaterials such as CNTs and graphene are also commonly used. The nanomaterial also serves as a sensing transducer on the working electrode in addition to serving as a conductive path, providing a large surface area for conjugating with bioreceptors to obtain high sensitivity. It is noteworthy that the nanomaterial inks must be well-dispersed in the solvent in order to avoid agglomeration and subsequent nozzle clogging. If necessary, the additives like surfactants can be added in the ink for stabilization.

Besides, the physical properties of inks (*e.g.*, viscosity, concentration and surface tension) influence printing performance. According to the working principle of inkjet printing, the applied ink must have relatively low viscosity, for it needs a sheer and thin texture for being easily propelled from the nozzle. The typical viscosity ranges from 10 to 20 cps. The limited viscosity determines that the inks for inkjet printing should not be concentrated, and the loading of fillers and binders should be low.<sup>90</sup> Another desired characteristic of the ink is low air-liquid surface tension. Since the orifice diameter of nozzles is small (20-50  $\mu\text{m}$ ), too high surface tension could result in difficulty for ejection.<sup>112</sup> Considering all the characteristics of inks, the evaluation for printability of one specific ink has been well-established based on the Weber number and Reynolds number. More information has been widely published and will not be introduced in this study.<sup>114</sup>

When the printed droplet contacts the surface of a substrate, the hydrophobicity of the substrate influences the pattern resolution. The diameter of the deposited ink droplet on the substrate is correlated with the footprint resolution, which is highly influenced by the wettability. Generally, a high contact angle facilitates an increased footprint resolution; vice versa, a low one induces a worse resolution.<sup>114</sup> The contact angle is determined by three aspects: the surface energy of the substrate ( $\gamma_s$ ), the surface energy of the ink ( $\gamma_l$ ) and liquid-solid interfacial surface energy ( $\gamma_{ls}$ ) as the Young Laplace equation described (Figure 1.2.13a).<sup>93,115</sup> Hence, in order to achieve good printing performance, besides modulating the ink formulation, optimizing the substrate is also effective. For example, different substrates have different surface energies which can be found in the book written by Katsuaki Sugauma.<sup>93</sup> Additionally, plasma treatment is practicable to tune the surface energy of a specific substrate. For example, plasma cleaning can create a high-energy surface, resulting in a smaller contact angle and more wettability. In contrast,  $\text{CF}_4$  plasma treatment creates a low-energy surface through the outermost fluorinated layer.

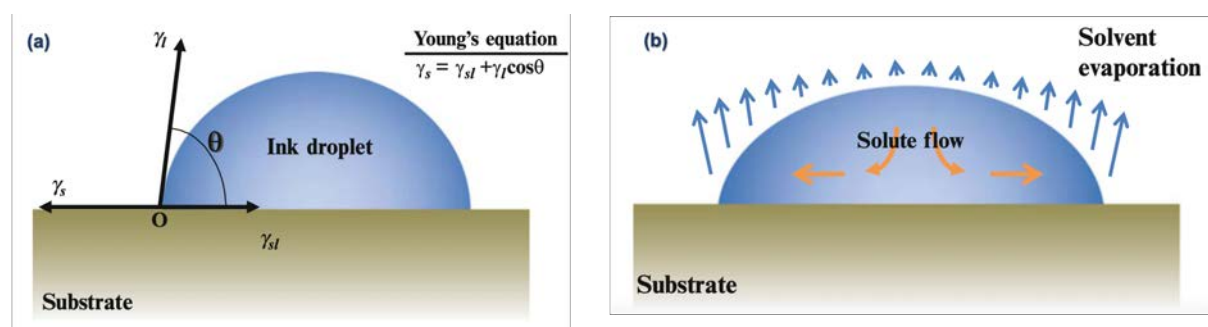


Figure 1.2.13 Illustration of (a) Young Laplace's equation with the abbreviation of the surface energy of the substrate ( $\gamma_s$ ), the surface energy of the ink ( $\gamma_l$ ), liquid-solid interfacial surface energy ( $\gamma_{ls}$ ) and contact angle ( $\theta$ ) and (b) the coffee ring effect.<sup>93</sup>

When the ink evaporates on the substrate, the surrounding condition also influences the resolution of printed patterns. Dispersed ink materials tend to deposit on the edge of evaporating droplets naturally, which is known as the "coffee ring" effect. It is caused by differential evaporation rates across the droplet, leading to an outward capillary flow. (Figure

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1.2.13b) The phenomenon worsens the printing quality and influences the resolution, resulting in printed sensors' malfunction. Hence, several approaches have been explored to suppress this coffee stain effect, such as cooling substrates, increasing humidity, and adjusting the pH of droplets.<sup>93</sup>

To summarize, the inkjet printing technique can highly reduce the wastage of (ink) materials and increase the speed of iteration with feasible modification of printing designs. Generally, its resolution can reach 20-50  $\mu\text{M}$  with a thickness below 500 nm (but the resolution of inkjet printing is also different from one printer to the other from a practical point of view).<sup>116,117</sup> Besides, compared to screen printing, fabrication by inkjet printing is considered relatively rapid.<sup>93</sup> It is important to balance resolution, fabrication speed, and printed film quality depending on the end-use application. For example, in the case of printing conductive inks, the priority is to obtain a continuous trace with low resistance. With this aim, the footprint resolution can be traded-off to guarantee more overlapping area between deposited droplets on the substrate, or the printing time can be extended to print multiple layers. Additionally, the good chemical stability of inks for long-term storage is preferable for inkjet printing.

Apart from the parameters discussed, printing equipment also determines the final printing performance. Besides the professional ones in the laboratory (*e.g.* Dimatix Materials Printer from FUJIFILM, DMP),<sup>92</sup> engineered office printers have been reported to fabricate low-cost electrochemical sensors, too.<sup>118,119</sup>

The professional printer features a good resolution and can print delicate and precise patterns *via* manipulating several parameters, *e.g.*, jetting frequency, drop spacing and applied voltage. It is also equipped with a camera to monitor the alignment, which allows to print the layered structure with different inks to construct fully printed electrochemical sensors.<sup>92</sup> The commonly used inks include conducting polymers, metals, metal oxides, ceramics and even biomaterials such as proteins and nucleic acids, *etc.*<sup>100,120</sup>

Taking the DMP as a typical example, Figure 1.2.14 demonstrates the procedures in one printing period of this printer. Drop formation and ejection are driven by piezoelectric actuation. The applied voltage performs the waveform of four stages with time. Each segment controls three properties: the duration time of applied voltage, the magnitude of applied voltage, and switch rate which is the slope of varied voltage vs time which determines how fast the piezoelectric transducer bends.<sup>92,121</sup>

In Segment 1 in Figure 1.2.14, the preparation step starts from the end of ejection (Segment 4) with the same standby voltage. In Segment 2 (Phase 1), the voltage is reduced to zero and renders the piezoelectric transducer to recover its shape. Meantime, with the pressure being released, the ink in the cartridge spontaneously fills in the channel. In Segment 3 (Phase 2), the voltage is highly increased and the transducer compresses suddenly. The ink in the channel is driven by the transient compression and steered from the nozzle. In Segment 4

(Phase 3), the voltage is decreased to the standby voltage and the transducer is partially recovered, which forces the ink to flow inward and pinches the droplet. Then, the voltage remains at the standby voltage and is ready for the next cycle. In fact, Segment 1 and Segment 4 are connected, and they are demonstrated separately just for a better understanding.

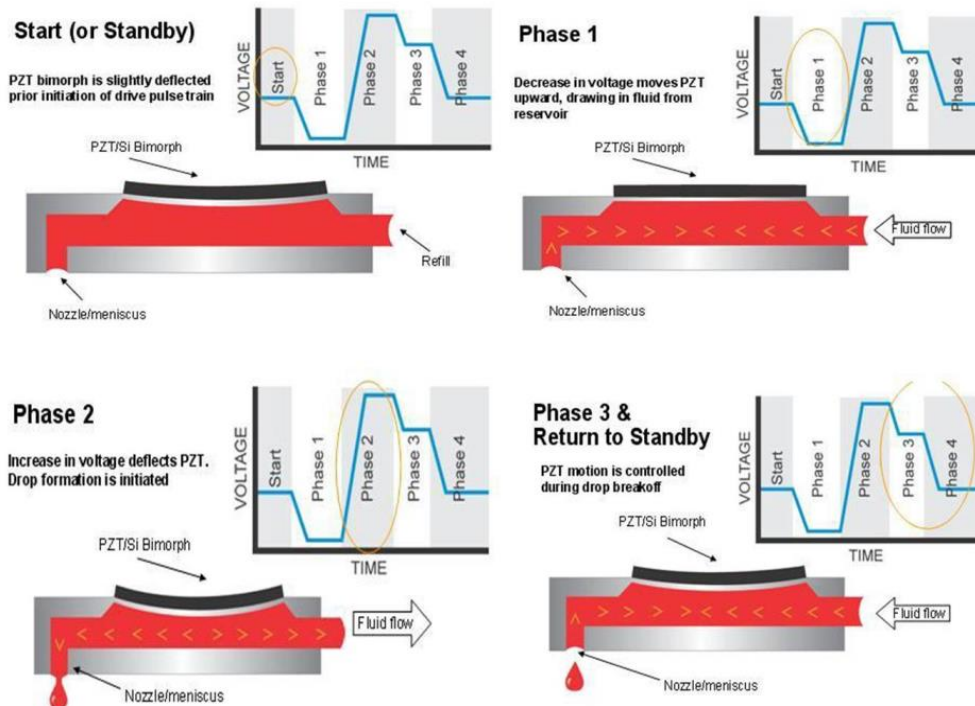


Figure 1.2.14 Working principle of DMP when ejecting ink droplets

Besides programming the voltage in the four segments, tuning the drop spacing in DMP influences printing performance. A high drop spacing can form single droplets on the substrate without any overlapping in Figure 1.2.15a.<sup>122</sup> Upon decreasing drop spacing which is slightly smaller than the diameter of the deposited droplet, a line of liquid beads with a periodic irregularity occurs (Figure 1.2.15b). When the drop spacing further decreases as small as the radius of the deposited droplet, the straight line can be printed (Figure 1.2.15c). However, if the drop spacing is too small, bulged parts with excessive fluid appear to spread in the unwanted area (Figure 1.2.15d). Particularly, when aiming for printing conductive paths, small drop spacing can be preferred to ensure the conductivity but good resolution can be also optimized by manipulating the wettability.

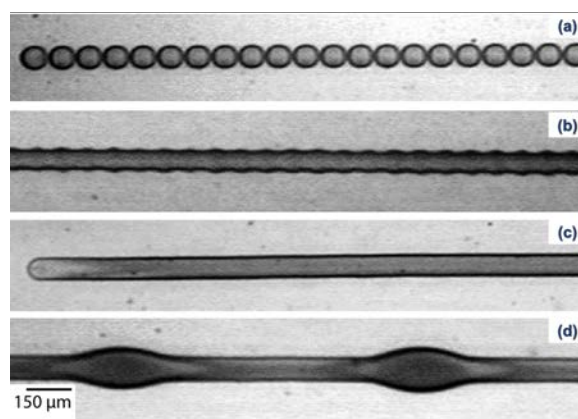


Figure 1.2.15 Printed traces by the drop spacing decreasing from (a) to (d).<sup>122</sup>

The professional (high-end) printing equipment is still limited by some issues even though it can control every drop on demand. Firstly, the nozzle clogging disturbs the printing process and poses a risk of damage to the clogged nozzles or even other nozzles on the same printing head during washing. The damaged nozzles could cause material wastage and delay fabrication time. For example, DMP only has 16 nozzles on one printing head, and if clogging occurs, the working nozzles have to print more paths to compensate for the loss, which consumes more time than expected. Second, it is difficult for untrained personnel to operate the professional inkjet printer. It cannot realize the scenario of easy fabrication in the office or at home. Third, given that the ink concentration is rather low, printing with a professional inkjet printer on a drop-by-drop basis may be time-consuming when building conductive or dielectric layers. Hence, the professional printer can have better performance to dispense functional materials (*e.g.*, sensing transducers, bioreceptors) on sensing electrodes, which only requires a very small quantity but makes a significant difference in the rapid and mass-produced electrochemical sensors.

Complementary to high-end printers, office printers are emerging to fabricate electrochemical sensors.

Office printer features high printing speed due to the numerous nozzles in the printing head and extremely short time when dispensing inks. Taking a commercial HP thermal printer as one example, a printing head has approximately 21,000 nozzles (Figure 1.2.16a-d).<sup>118</sup> Thanks to the great number of nozzles, the commercial printer can emit ink precisely with the jetting frequency of more than 40,000 times per second, which means within 50  $\mu\text{s}$ , one nozzle completes one period of ejecting (Figure 1.2.16e-h). Several cartridges filled with different-color inks provide a possibility to print several stacked layers with one path to prevent alignment issues within a short time. Accordingly, it features high fabrication speed and mass production. Besides, the office printers have been already equipped with nearly all the printing elements (*e.g.*, printing head, cleaning pad, and cartridges) without requiring any personnel for installation. It facilitates the easy fabrication of electrochemical sensors. Most importantly, the office printer is beneficial in low-budget circumstances compared to high-

end printers. Besides original inks, office printers could also print customized inks. Theoretically, the synthesized ink can replace the original ink in the printer cartridge if they have similar ink characteristics (such as surface tension, particle size, and viscosity).

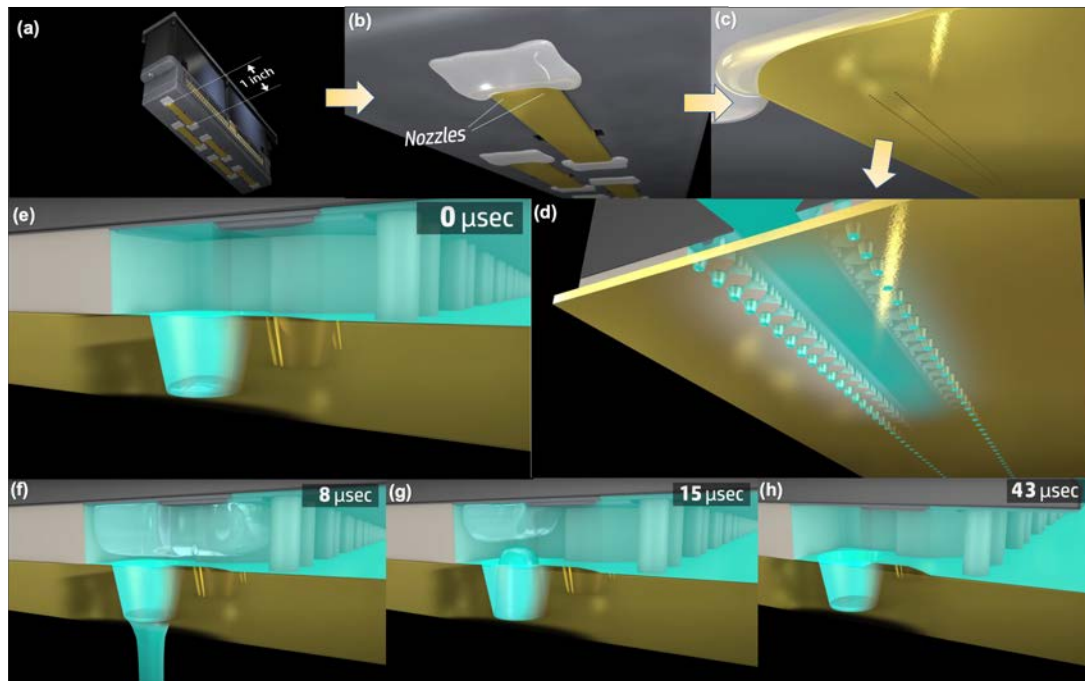


Figure 1.2.16 Schematic illustrations of a printing head in a commercial inkjet printer (HP Thermal Inkjet printer). (a) Illustration of the printing head, (b) enlarged vision of silicon die on the printing head, (c) two rows of nozzles (zoomed) on the silicon die, and (d) the structure of nozzles with ink inside (blue). (e)-(f) Thermally controlled bubble propelling ink droplet with time in one period within 50  $\mu\text{s}$ .<sup>123</sup>

However, cross-contamination hurdles the progress of the office printer fabricated sensors. Since office printer typically works under standard CMYK mode, meaning four original colors (*i.e.*, cyan, magenta, yellow and black), are blended to create the rest of the colors, it poses a risk that one certain nozzle may not corresponding to one certain color. As such, the inks that remained in the printing channel could induce cross-contamination when printing another layer. The desired ink can be blended with dye impurity, causing unexpected chemical contamination. Fortunately, there has been some published study noticing this phenomenon and offering the methodology to avoid cross-contamination.<sup>118,125</sup>

Another issue for office printers is the limitation of *in-situ* curing, inducing the limited selection of substrates. Unlike high-end inkjet printers equipped with accessories of a UV light and a heating panel to cure the ink deposited on the substrate, office printer is limited to further engineering. Besides, the office printer needs to roll the substrate, then print patterns, and lastly push it out of the printer, and the printed pattern without curing is still in the liquid phase and frequently ruined by any accidental movement. It causes unwanted spreading and spilling. In this perspective, porous substrates seem useful, for their porous structure can absorb the excessive ink solvent instantly and leaves the solid nanomaterials to be deposited

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on the surface. Papers, with a natural ability to wick fluids and biodegradability, have been used as absorbent substrates to fabricate electrochemical sensors. For example, a paper-based electrode printed with AuNPs has been reported to be used as a sustainable electrochemical sensor to detect glucose in blood serum.<sup>125</sup> In this study, starch, as an effective additive in inks, was used to tune the rheological properties with the assistance of heating. However, the annealing post-treatment was necessary to achieve good conductivity of printed electrodes. This treatment always renders paper more brittle than normal, which limits the use of flexible and long-term monitoring sensors.

A special porous substrate is developed to address the issue of curing metallic inks at room temperature. Metallic nanoparticles dispersed in the inks are typically required to be capped with ligands or surfactants (mostly nonionic type) for stabilization because these nanoparticles have high surface energy giving rise to easy aggregation.<sup>118,125</sup> This approach also allows a high metal loading in the ink. However, printed paths are not conductive yet due to the capped ligands should be removed to ensure conductivity. Unlike the conventional method to remove the ligands at a high temperature, the special substrate has an adsorbent pad (porous resin) on top, where chemical sintering agents (*e.g.*, NaCl) are pre-stored.<sup>113</sup> When the ink is printed on the adsorbent pad, the ink solvent penetrates the adsorbent pad and releases the stored reagents so as to remove the ligands and induce the nanomaterials to aggregate.<sup>125</sup> Hence, this curing process allows high conductivity at room temperature. Besides, this substrate and the nanoparticles inks are commercially available, but solely limited to AgNPs offered by Mitsubishi Paper Mills Co., Ltd and Novacentrix Co., Ltd.<sup>125–127</sup>

Taking advantage of office printers and associated commercial products, we reported a Ag interdigitated nanoelectrode manufactured by an office printer for the diagnosis of early-stage kidney injury *via* detecting its biomarker (neutrophil gelatinase-associated lipocalin, NGAL). In this work, a robust fabrication has been established successfully by an office printer with the commercial ink and substrate as aforementioned. The resolution of inline and outline printed paths was investigated. Based on it, the interdigitated electrodes were printed. However, the adsorbent pad beneath the electrode caused a drawback. For the electrode resides on top of the adsorbent, a hollow structure was created beneath the electrode, which causes leakage during conjugating and testing. To address this bottleneck, wax was utilized to print on the surface of the electrode and heated to fill the pores of the adsorbent pad. Then, the aptamers were directly modified on the Ag nanoelectrode as bioreceptors *via* the bond of Ag-S. When NGAL existed in the tested solution, the impedance of the electrode was increased compared to the counterpart in a blank sample, without requiring any labels. Finally, a mobile-phone based readout was integrated in the sensing system to realize point-of-care applications.<sup>125</sup> This study demonstrates great potential to fabricate mass-productive and cost-effective electrochemical sensors by office printers, although some issues are remained to be solved. For example, the wax filling strategy renders the substrate more brittle, and an additional heating process poses the risk of substrate deformation. Despite this, it offers a



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promising scenario where people may design and fabricate customized sensors by themselves depending on their specific needs in the future.

#### 1.2.2.4 Wax Printing

Besides filling the adsorbent pad of office printing electrodes, wax printing is more exemplary to define the fluidic channels *via* its hydrophobic nature (Figure 1.2.17). With the sparked substantial interest in *in-situ* measurements, manipulating tested fluids becomes important to realize multiple functions in one electrochemical sensor/sensing system. This trend facilitates the use of wax printing to proliferate. Commercial printers (ColorQube 8880 Series from Xerox Holdings Corporation) can print patterns of solid wax on paper with solid ink with high precision and sustainability. The printing speed is also high to print one A4-sized document in approximately 5s, which is promising for mass production.<sup>128</sup>

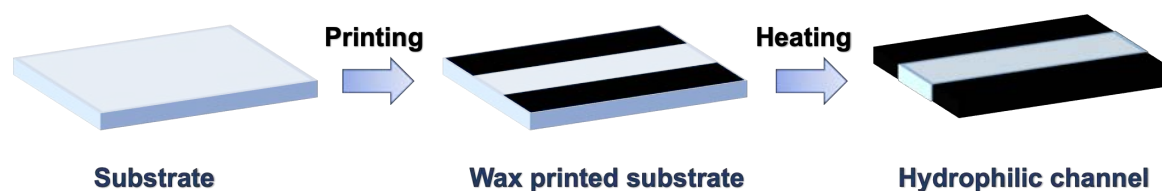


Figure 1.2.17 Schematic illustration of wax printing applied in the fabrication of microfluidic channel.

In 2009, Lu et, al. firstly utilized the wax printing technique to create a hydrophobic pattern on papers and compared wax printing with other techniques printing photoresist and polydimethylsiloxane (PDMS). Wax was firstly printed on the top of a paper, and then, printed wax melted, filled the pores of cellulose, and penetrated the full thickness of the paper by a heating process. After the temperature cooling down, hydrophobic wax boundary and hydrophilic channel were formed. Wax printing showed faster, cheaper, and easier fabrication compared to the other two techniques, without requiring other additional equipment and trained personnel.<sup>129</sup>

Besides, wax printing is exploited to fabricate sensing electrodes, in which a hydrophilic membrane is first patterned, followed by a heating procedure to melt the wax and create a hydrophobic barrier. Then, an ink containing the well-dispersed electrode is filtered by this membrane, on which the sensing material can only deposit on the top of the uncovered part. Lastly, the deposited sensing material with a specific pattern can be transferred to the desired substrate by stamping.

As such, our group presented a graphene oxide (GO) working electrode thanks to the introduced strategy. GO aqueous solution was filtered on the patterned membrane and then stamped on the commonly used substrates such as PET, polyimides (PI), and polyethylene naphthalate (PEN). This approach reached a resolution down to 50  $\mu\text{m}$ .<sup>130</sup> Then, this technique was improved to fabricate a rGO biosensing electrode. AuNPs conjugated with DNA

probes were mixed with rGO to form a water-based ink. The formed agglomerates were sonicated and showed stable dispersions in water. After filtering and stamping, the composite formed the rGO sensing electrode with DNA probes on the surface (Figure 1.2.18). It can detect Coxsackievirus B3 whose infection could induce myocarditis.<sup>131</sup>

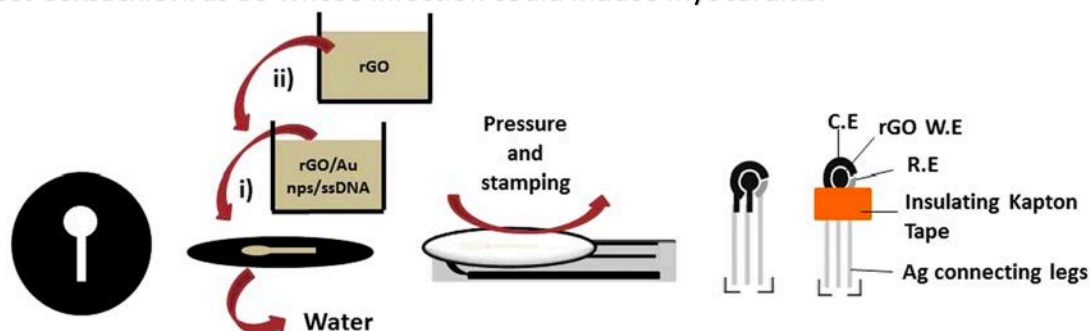


Figure 1.2.18 Scheme of the fabrication of working electrode by wax printing and filtration.<sup>131</sup>

Wax stamping is considered more eco-friendly owing to its typical cellulose-based substrates. Besides, the free use of solvent additives and stabilizers can enhance conductivity and simplify post-processing. However, this patterning technique also has challenges to face, for example, the filtered inks should not dissolve the membrane material. Despite this, with adequate optimization of the ink formulation, a wide range of nanomaterials could be adapted.

Overall, the wax printing technique is highly useful for the manufacture of microfluidic devices and nanomaterial-based sensing electrodes with the aim of eco-friendly fabrication. However, in 2016, the exemplary commercial wax printer—ColorQube 8880 series is discontinued. To address it, some studies attempted to substitute for the printer and wax, but the altered wax cannot penetrate thoroughly and required a longer heating time than the commercial one.<sup>128</sup> New techniques to replace wax printing are highly demanded at this stage.

### 1.2.2.5 Other Printing Techniques

Roll-to-Roll (R2R) Gravure printing is typically used to pattern inks on the flexible substrate based on the patterned slot-die on the roller. Inks only fill the slot, and the rest is removed by a blade (Figure 1.2.19). Then, the ink in the patterned slot is transferred to a flexible substrate thanks to the pressure offered by rolling cylinders. As such, R2R gravure printing has high process speed and high-throughput fabrication. R2R gravure is also considered to have a faster printing speed with superior resolution and consistency over screen printing in the fabrication of sensing electrodes.<sup>132,133</sup>

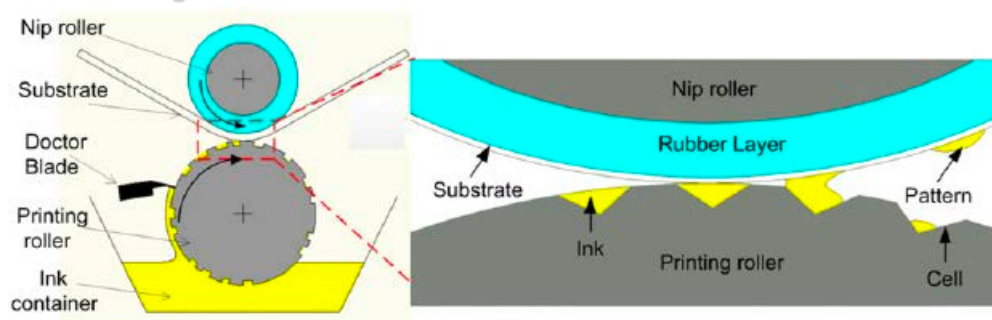


Figure 1.2.19 Schematic of the R2R gravure printing process: overall configuration of printing process between printing and nip rollers, and detailed view of the contact point.<sup>134</sup>

Beside R2R gravure printing, laser-assisted patterning techniques have been utilized in the fabrication of sensing electrodes.

A laser is able to cut film-like material, and thus one typical fabrication way is to shape electrode material by cutting with the desired pattern. For example, Alireza et al reported the laser-shaped nanosilver-coated copper tape as an electrode to detect glucose based on its electro-catalytic activity toward glucose oxidation.<sup>134</sup> Besides, laser cutting is exploited to shape a plastic film by cutting and removing unwanted parts to create a mask for the following steps. When depositing sensing materials with the mask, electrodes with specific patterns can be fabricated (Figure 1.2.20a). This approach is more compatible with versatile sensing materials compared to the previous strategy. For instance, as reported by Alyssa et al., a glassy carbon microparticle electrode was printed with the assistance of a laser-cutted mask by stencil technique.<sup>134</sup> Unlike the complex fabrication of traditional glassy carbon electrodes (GCE), this sensor can detect the heavy metal ions (HMIs), namely  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  ions with good sensitivity, balancing the manufacturing overhead.

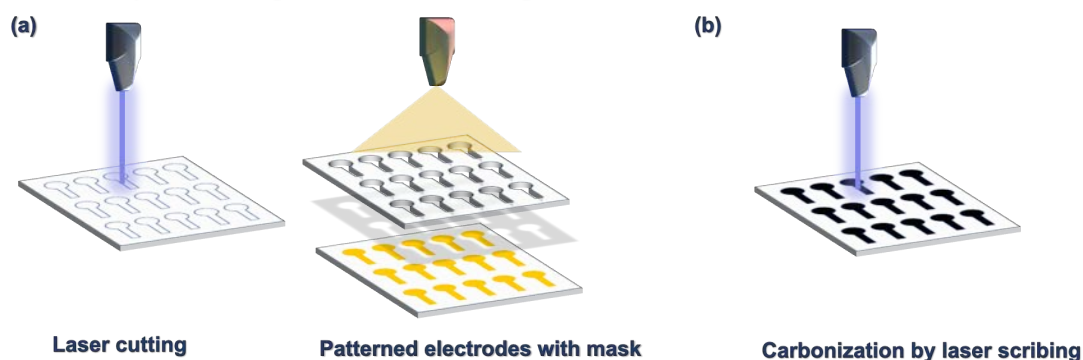


Figure 1.2.20 Electrode fabrication by (a) laser cutting and (b) scribing

Additionally, direct laser scribing (so-called ‘engraving’) can be also used to fabricate carbon-based sensing electrodes. Laser-scribing PI film is a typical way to fabricate porous graphitic carbon electrodes.<sup>135–137</sup> This technique does not strictly require the wavelength of laser light, for the visible laser light can induce the carbonization of PI, which could lower the cost of equipment ownership.<sup>138</sup> More surprisingly, besides PI, other common polymeric substrates (*e.g.*, PDMS, polyether ether ketone) and some biomasses such as coconut shells, bread *etc* can be converted by laser irradiation to substitute for the expensive PI substrates.<sup>139–141</sup> Yet these alternative materials have rarely been tested as electrochemical sensors. Moreover, laser scribing can realize the one-step synthesis of new composites such as heteroatomic atom doped graphene, and graphene functionalized by metal/metal oxide nanoparticles (*e.g.*, Cu and ZnO) *via* the addition of metallic precursor in the polymer substrate.<sup>137,142,143</sup> However, few studies about these composites have been applied them in electrochemical sensors in the environmental monitoring and healthcare fields.

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Apart from these alternatives, GO film is used to fabricate laser-scribed graphene electrodes. However, the remaining GO on the substrate could interfere with the electrical signal in water-based samples because GO can be dissolved in water. To deal with this issue, our group established a novel stamping technique,<sup>144</sup> in which only the laser-scribed rGO was transferred from the GO film to the surface of other desirable substrates such as paper and PET selectively. The transfer of rGO was realized by stamping under pressure due to the discriminated surface affinity of laser-scribed rGO and GO with desirable substrates. As proof of concept, the electrochemical performance was examined by CV on the laser scribed rGO, which was transferred on PET and nitrocellulose as WE.

Overall, traditional printing techniques such as screen printing and R2R gravure techniques can be compatible with many commercial inks, featuring high process speed and mass production. Screen printing can fabricate thick films in one path which is quite challenging for other printing techniques. Emerging techniques like inkjet printing and laser-patterning techniques are more specialized in high precision and depositing thin films with customized inks or precursors. Hence, to take advantage of various merits of printing techniques, the combination of these techniques is frequently found in electrochemical sensors, which balances the process speed/simplicity and printing performance.

These printing techniques could facilitate the dissemination of sensors compared to the conventional microfabrication techniques, which can be helpful to construct sensing networks to monitor environmental pollutants and certain diseases, especially in resource-limited regions.

With the increasing demand for sensors with high sensitivity and selectivity, nanomaterials have been employed as a new strategy to enhance the sensing performance of electrochemical sensors due to their high surface-to-volume ratio and catalytic properties. Particularly, due to good mechanical properties, nanomaterials are also applied in flexible, stretchable and even wearable sensors, which are highly useful in monitoring important biomarkers in the human body. Hence, in the next subsection, the types of nanomaterials and their properties will be introduced.

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## 1.2.3 Nanomaterials for Signal Enhancement in Electrochemical Sensors

### 1.2.3.1 Definition and Features

Nanomaterials are defined as materials with an external dimension or internal structure in the nanoscale range (less than 100 nm). The surface-to-volume ratio is enhanced with the decrease in size and more atoms exposed to the surface. Therefore, the surface properties of the nanomaterials are more apparent, expressing different and unique properties when compared to their bulk counterpart. An intuitive example is AuNPs which can be suspended in water uniformly due to the large surface with high surface energy, but its counterpart, bulky gold, is impossible to do so. Besides, at nanoscale, quantum confinement of the charge carriers induces changes in the electronic structure of the nanomaterials, opening a path for investigating new optical-electronics materials (*e.g.*, quantum dots).<sup>145</sup>

As such, some properties of the nanomaterials, offer many advantages for their use in sensing applications. In this section, the most important chemical and physical properties of nanomaterials are highlighted.

#### 1) *Surface-to-volume ratio*

Most evidently, the decrease in the size of nanomaterials leads to an increase in the surface-to-volume ratio which exposes more atoms to the surface and renders the surface more reactive to the surrounding environment.<sup>146</sup> The surface allows for a higher specific area for conjugation with more receptors and provides more active sites. This effect greatly enhances the efficiency of chemical/biological recognition and reaction.<sup>147,148</sup> On the other hand, the nanosize can also prove to be potentially toxic. In particular to wearable sensors where these devices are in direct contact with the skin surface, the effect of toxicity cannot be ignored. Much progress has been made so far in the study of the biocompatibility, cytotoxicity, and biodegradability of the nanomaterials; however, these materials are still under careful evaluation.<sup>149–152</sup>

#### 2) *Electrocatalysis*

Catalysis plays an important role in the selectivity of sensing devices, for it is a process of increasing the rate of a specific chemical reaction. Because of the large fraction of atoms exposed on the surface, nanomaterials have more active sites and, thus, can be well suited as highly efficient catalysts such as metallic nanoparticles, metal oxides, zeolites, and carbon nanomaterials. In terms of (bio)chemical sensing, a purpose of such nanomaterial catalysts is to substitute for the enzymes in enzyme-based sensors, as they can be expensive to purchase and require very delicate handling. The most common approach is to integrate these catalysts working as the label to trigger the reaction with the substrate by monitoring electron transportation.<sup>153</sup> On the other hand, catalytic nanomaterials can be employed in the development of electrochemical biosensors, decreasing the overpotential necessary for an electrochemical reaction to occur and increasing the output signals.

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### 3) *Electronic characteristics*

The electrical conductivity of bulk metals is influenced by their electronic band structures and the mobility of electrons within the lattice. In nanomaterials, however, the phenomenon is different as the electronic energy levels are not continuous but are discrete (finite density of states). As such, the effects of quantum confinement are more apparent and dictate the electronic behaviors in the metallic and semiconductive nanomaterials, and give rise to unique electronic properties not observed in the bulk.<sup>154</sup>

Another advantage is the high electrical mobility of nanomaterials, especially in low-dimensional nanomaterials (one-dimensional (1-D) and two-dimensional 2-D) due to their confined size limit.<sup>155,156</sup> This is an important characteristic, especially for FET-based sensing devices as it affects the carriers' mobility rates and increases the conductivity and switching speed. For example, due to the distinct band structure and physical structure of the graphene and CNTs, the mean free path of their charge carriers is high, making it possible for the charge carriers to move by microns (0.3  $\mu\text{m}$  for graphene) in the surface of these materials before a scattering process occurs.<sup>157</sup> This contributes to the high mobility values of the charge carriers in these nanomaterials (15,000 and 79,000  $\text{cm}^2/\text{Vs}$  at room temperature for graphene and semiconducting CNTs, respectively),<sup>157,158</sup> improving the electrical transport and the performance of the sensing devices based on these nanomaterials. Besides, the high electrical conductivity of nanomaterials with metallic behavior, such as AuNPs, silver, and gold nanowires, metallic CNTs, and even graphene (a semiconductor with a zero-band gap) highly improves the electrical sensitivity of the interactions of the target molecules on the surface of these nanomaterials, leading the development of (bio)chemical sensors able to achieve lower LODs.<sup>159,160</sup>

### 4) *Magnetic properties*

Due to the small dimension, the increased surface area promotes the magnetic coupling with adjacent atoms, which manifests the diverse magnetic properties of several nanomaterials. The magnetic properties are used in a variety of applications such as ferrofluids, drug delivery, catalysis, and magnetic memory storage devices.<sup>161</sup> In particular to biosensing, their small size, and large surface area are typically harnessed, and it is utilized as a transporting tool to carry and accumulate receptors and targets, which enhances the sensing signals.

### 5) *Mechanical properties*

Nanomaterials show superior mechanical properties in terms of hardness, elastic modulus, and fatigue strength compared with rigid bulk materials. For example, graphene presents with a record strength (130 GPa) and Young's modulus (1 TPa).<sup>162</sup> Besides, due to the unique physical structure, the 1-D and 2-D materials can be stretchable and bendable, but without drastically altering their electrical conductivity, preventing the delamination, and/or the

cracking process of the activity under large mechanical strain in the wearable sensors, which is a key requirement in the design of materials for e-skin devices and sensors.<sup>163,164</sup>

### 1.2.3.2 Classification and Examples (0-D/1-D/2-D/3-D Nanomaterials)

Due to the complexity of nanomaterials, we organized and classify the nanomaterials as presented in Barhoum et al.'s book as (i) 0-D nanomaterials (0DNMs), (ii) 1-D nanomaterials (1DNMs), (iii) 2-D nanomaterials (2DNMs) and (iv) 3-D nanomaterials (3DNMs).<sup>165</sup> Some popular examples are shown in Figure 1.2.20.

#### 1) 0-D nanomaterials (0DNMs)

0DNMs are defined by dimensions that are less than 100 nm and can include morphologies such as metallic nanoparticles /nanoclusters, metal oxide particles (such as transition oxide nanoparticles), magnetic nanoparticles, carbon dots, fullerene, and quantum dots (Figure 1.2.21). These materials possess features such as photoactivity, high efficiency for catalysis (owing to their high curvature of the nanoparticles' surfaces), more surface defects, and quantum electronic properties. Thus, they can be applied to electrochemical sensing platforms to enhance their sensitivity.

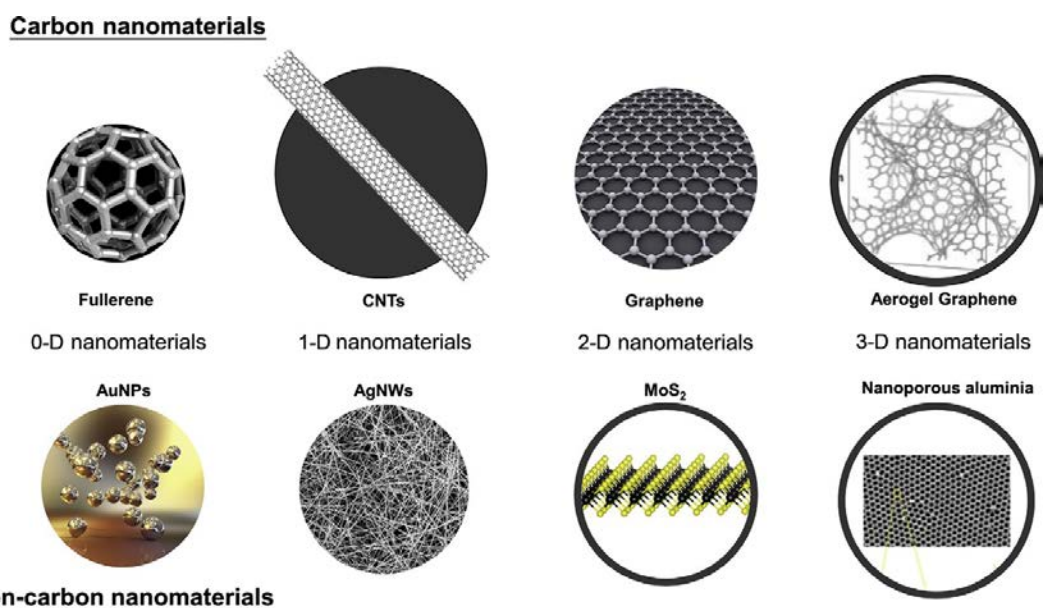


Figure 1.2.21 Dimensional classification of nanomaterials.<sup>166</sup>

A widely researched and used 0DNM is AuNPs, which have several advantages for signal enhancement in chemical and biological sensing. AuNPs can be synthesized by a simple method and are typically stable in aqueous environments. The high surface-to-volume ratio provides abundant active sites to be conjugated with a wide range of biomolecules, such as ligands, proteins, nucleic acids, etc., for single or multi-analyte detection. By harnessing ease of functionalization nature of AuNPs, enhancement of the sensing signal can be achieved through efficient binding of analytes to receptors on the AuNPs, which has been widely proved in other sensing platforms such as surface plasmon resonance, enzyme-linked

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immunosorbent assay, and LFA devices.<sup>167</sup> AuNPs also play an important role in electrochemical sensing platforms as labels due to their superior electrocatalytic properties, which induce the drastic change in electron transport during a catalysis event.

Moreover, magnetic ODNMs have been widely employed as nanocarriers of acceptors to accumulate sensing targets and enhance sensing signals. In this sense, the work conducted by Zamora-Galvez et al. explored very well these features.<sup>168</sup> The authors developed a nanocomposite based on molecularly imprinted polymer (MIP)-decorated magnetite nanoparticles to separate, preconcentrate and manipulate the sulfonamides (the analyte) which are selectively captured by the MIP onto the surface of the composite, in screen-printed electrochemical sensors. Combining EIS-based detection, the high surface area of the magnetic nanocomposite and its extraordinary ability to pre-concentrate the analytes, the MIP sensor was able to detect impressive levels of sulfonamides, showing an extremely low LOD of  $1 \times 10^{-12}$  mol/L ( $2.8 \times 10^{-4}$  ppb).

Additionally, ODNMs can also directly react with the sensing target. Bujes-Garrido et al. reported a simple electrochemical wearable sensor to detect  $\text{Cl}^-$  ions, an important biological indicator to monitor electrolytes in the human body, with AgNPs and screen-printed voltammetric sensors.<sup>169</sup> By harnessing the chemical reaction between the  $\text{Cl}^-$  ions and the AgNPs, the electrons generated from the reaction (electrical output) could be monitored. This disposable and wearable sensor was then applied for the detection of  $\text{Cl}^-$  ions in sweat. As the ODNMs have a larger surface-to-volume area than their bulk, more active sites are accessible to drive the reaction.

## 2) 1-D nanomaterials (1DNMs)

1DNMs are considered as nanomaterials that possess dimensions where one dimension is significantly larger than the other two in the nanoscale range. Based on this definition, 1DNMs are typically nanowires, nanotubes, and nanofibers. Due to the unique structure and high surface-to-volume ratio, 1DNMs are applied to electrochemical sensors which have been shown to be beneficial for signal-enhancing purposes.<sup>170,171</sup> As the electrons are confined to the 1-D structure, the conductive nature of the nanomaterial remains. In particular, the wire-like structure can easily form a percolation network that is beneficial for stretchability and flexibility in wearable sensors in the healthcare field. Also, as the electrons are confined to the 1-D structure, the conductive nature of the 1DNMs remain. Thus, 1DNMs are promising candidates in wearable sensing, compatible with FETs to achieve higher sensitivity.<sup>172</sup> Moreover, in comparison to other nanomaterials, 1DNMs are also superior in transparency without much trade-off in the other properties.<sup>163</sup>

CNTs, as a typical example of 1DNMs, have well-ordered, internally hollow, and long cylindrical structures composed of  $\text{sp}^2$ -hybridized carbon atoms (a rolled-up graphene sheet). CNTs are typically found in two types: single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs). The CNTs can show either a semiconductor or metallic behavior that is related to



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their diameter and chirality. The chirality of the CNTs depends on how the graphene sheet is oriented on rolling, which is specified by a vector (called a chiral vector), so the CNTs can be armchair, zig-zag, and/or chiral. In this way, the armchair CNTs share electrical properties similar to metals, which include the MWCNTs; and the zigzag and chiral CNTs possess electrical properties similar to semiconductors, which include the SWCNTs.<sup>173</sup> Depending on their electrical properties, a specific type of CNTs can be chosen for the design of the electrochemical sensors. For example, MWCNTs due to their metallic properties are good candidates for the development of a working electrode in electrochemical sensors; in its turn, the semiconductor SWCNTs are indicated for the development of FET-based sensors. Like ODNMs of the general high surface area, CNTs can provide a large surface for conjugation with receptors and targets, and thus provide high efficiency for mass transport of analytes to enhance the sensing signal.

For example, Bandodkar et al. constructed a CNTs-based electrochemical sensor and biofuel cell.<sup>168</sup> Thanks to the superior mechanical resistance of CNTs, the sensor could sustain strain as high as 500% without influencing the sensing performance. To link the receptor to the surface, the CNTs were functionalized with selective ionophores and enzymes with the interest to detect a wide range of analytes. This wearable sensor was tested to detect ammonium ( $\text{NH}_4^+$ ), as a model, by potentiometry. Taking advantage of the biofuel concept, where glucose was both the analyte and the biofuel, the enzymatic reaction was utilized to generate changes in the current that was monitored by amperometry. Consequently, the sensor could operate in a self-powered mode without an external power source.

### *3) 2-D nanomaterials (2DNMs)*

2DNMs have one external dimension in the nanoscale range and the others are significantly larger, comprising nanosheets and nanofilms. In this system, electrons are confined within one dimension, indicating electrons cannot move freely within the associated dimension. The atomic thickness offers them high mechanical flexibility, optical transparency, a large basal surface, and tunable conductivity for the conjugation of bioreceptors, which makes them a promising material for the fabrication of electrochemical sensors.

Amongst them, graphene has attracted considerable attention since its discovery in 2004, owing to its remarkable electrical, optical, mechanical, thermal and biocompatible properties. The idealized graphene is completely two-dimensional, comprising a single layer of  $\text{sp}^2$ -hybridized carbon atoms joined by covalent bonds to form a flat hexagonal lattice. Graphene has outstanding physical properties including high carrier mobility and capacity, highly tunable conductance and exceptional mechanical strength and flexibility; it is therefore very useful in science and technology.

In recent years, graphene-based nanomaterials become an excellent sensing platform for carrying active probes and molecules of interest, which is beneficial for the construction of novel (bio)interfaces for biosensing applications. Besides, graphene derivatives generally

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prove to enhance the electroactivity of the WE, and thus, to modify graphene derivatives on the sensing electrode is a widely used enhancement strategy for electrochemical sensors. In an interesting study, Mannoor et al. presented a sensor that is wearable on tooth enamel and was capable of wireless data transmission.<sup>174</sup> In this sensor, the transducer was selected as graphene printed onto silk in an interdigitated configuration. The transducer itself was then functionalized with antimicrobial peptides as receptors for selective binding to bacteria (*Helicobacter pylori*) in the saliva. Due to the large surface area of graphene, the signal was enhanced, and the sensor achieved an excellent LOD of  $\sim 100$  cells. The wireless communication module enabled the sensing system to communicate with a smartphone for quantitative analysis.

Particularly, graphene has been extensively explored in the development of high sensitivity biosensors based on FETs (gFETs),<sup>174–176</sup> by using the modulation of the conductivity in the gFETs as a function of the interaction between the target molecule and the bioreceptor, immobilized on the graphene surface. In this way, the gFETs are very appealing devices to be designed as highly sensitive electrochemical sensors without requiring any redox probes and labels for the detection of non-electroactive biomarkers (such as proteins) in the field of healthcare.

In addition to graphene, other graphene-like 2-D nanosheets, such as graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>), boron nitride, transition metal dichalcogenide (TMDs), and transition metal oxides, have received significant consideration in the past few years due to their features such as large surface area, excellent thermal conductivity and electric conductivity, and ease of functionalizing the surface.<sup>177</sup>

A vibrant research area on TMDs has emerged during the past decade, which represents 2-D layered materials with ultrathin structure. The properties of TMDs highly depend on the degree of crystallinity, number of layers and stacking sequences in their crystals and thin films. The molybdenum disulfide (MoS<sub>2</sub>) is the most popular member of layered TMDs and has withdrawn immense attention due to its extraordinary crystal, electronic and optical properties. It features a unique structure, where the layer of Mo atoms is arranged in a hexagonal array and sandwiched between two sulfur (S) layers. The Mo–S bond is held by a strong covalent bond while the Van der Waals interaction exists between S layers. MoS<sub>2</sub> semiconductors have recently attracted attention because of their outstanding mechanical and optical transmittance, high gauge factor, and tunable band gap in the function of the number of layers, which can exhibit high photoluminescence when found in a monolayer (direct band gap).<sup>178</sup> MoS<sub>2</sub> nanosheets own piezoelectric properties and have been explored as piezoresistive sensors, improving the sensitivity of these sensors and offering high operation stability.<sup>179,180</sup>

#### *4) 3-D nanomaterials (3DNMs)*

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3DNMs include nano-sized grains or nano-sized internal pores without the confinement in all external dimensions, with different shapes such as foams, sponges, hydrogels, and aerogels. To fully take advantage of the large surface area of 3DNMs for signal enhancement, the porous surface can be modified with bioreceptors and chemical catalysts like metal nanoparticles with improved loading density of these species.<sup>181–183</sup> The free-standing 3-D structure is also beneficial for introducing fluid and gas for contact with the surface. In some cases, such as heavy metal sensors, the 3-D structure can accumulate and capture more cations and enhance the signal. Moreover, 3DNMs have improved mechanical deformation behavior, which is promising in wearable sensors.

A novel example of combining the advantages of a large basal plane of 2DNMs and 3-D free-standing material can be seen in the work of Wang et al.<sup>184</sup> Here, the authors reported a novel flexible FET biosensor employing a transducer out of rGO encapsulated 3-D natural pollen for the selective and sensitive detection of prostate-specific antigen. Harnessing the large surface of the graphene-based 3-D structure, the sensitivity of the sensor was significantly improved, allowing the PSA detection down to  $1.7 \times 10^{-18}$  mol/mL.

### 1.2.3.3 Summary

Due to their interesting chemical and physical properties, including (1) high electrical conductivity, (2) high surface area and aspect ratio, and (3) electrocatalysis, nanomaterials can offer many advantages and opportunities for the development of electrochemical sensors over the traditional bulk materials. The enhancement strategies have been developed to improve the performance of sensors by using various nanomaterials. The ultra-high sensitivity of biochemical detection and other features of the low-dimensional materials appear as appealing advantages. Besides, nanomaterials can offer new opportunities for the implementation of electrochemical sensors based on various printing techniques, allowing to design of a whole (hybrid) printable sensor.

However, it is evident that most of the presented enhancement strategies still face several challenges that have hindered their translation and adaptation for real applications. Obstacles such as trade-offs in material properties and the high demand for simplicity, robustness, and design of sensing systems still need to be addressed. Besides, the enhancement strategies are mainly to take advantage of the large surface area provided by nanomaterials to support more receptors. However, this feature also induces potential toxicity of nanomaterials which is urgently demanded to be clarified by more experimental results than the presented ones nowadays.

Furthermore, we also found there is an increasing trend of electrochemical sensing studies using nanomaterials focusing not on the sole sensor itself but on the whole sensing system, including power management (or battery-free system), substrate material, and electronic circuit, and wireless communication module. Amongst, power consumption remains a key issue, and thus, self-powered, such as piezo- and biofuel-based, platforms will be more

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popular in the future. Instead of batteries, more different forms of energy will be involved such as radio-frequency identification (RFID), wireless charging, solar cells, and fuel cell.

#### 1.2.4 Overview of Chapter 1.1 & 1.2

Overall, electroanalysis, particularly the electrochemical sensor, simplifies the detection of ions, molecules and pathogens in the fields of environmental monitoring and healthcare, to respond to the urgent demand for *in-situ* sensing nodes. It fulfills the gap between the practical use for *in-situ* measurements and conventional laboratory-based analysis, which requires complex operation by trained personnel. With the purpose of fabricating mass-productive sensing nodes to spread their use, printing techniques have been explored, such as screen printing and R2R gravure. Emerging techniques, for example, inkjet printing, wax printing, and laser printing are also beneficial for fabricating cost-effective sensors with their own specific features. Combining these printing techniques, fully-printed sensing systems can be also achieved. Furthermore, facing the challenges of sensitivity and selectivity, nanomaterials have been widely harnessed to improve sensing performance. Especially, the mechanical properties of nanomaterials are highlighted, which allow them to be used in wearable sensors. These three strategies demonstrate great potential to fabricate simple, portable, cost-effective, user-friendly, and highly sensitive sensors for *in-situ* measurement in the environment and on the human body.

Heavy metal ions (HMIs)—typical pollutants in the environment and bio-indicators in the human body as well— are targeted as the main analytes in this study; thus, HMIs sensors will be introduced in the next section. State-of-art studies of *in-situ* sensing systems by the anodic stripping voltammetry (ASV) for environmental monitoring and wearable healthcare will be summarized. Moreover, interference problem, one of the most important open issues to be addressed in the ASV technique, will be discussed in the following section together with their associated strategies.