Organic Bioelectronic Devices for Selective Biomarker Sensing: Towards Integration with Living Systems

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*"Fatti non foste a viver come bruti,
ma per seguir virtute e canoscenza"*

*"You were not made to live as brutes,
but to follow virtue and knowledge"*

Dante Alighieri, Inferno

ABSTRACT

Inorganic materials have been the main players of the semiconductor industry for the past forty years. However, there has been a continuous interest and growth in the research and in the application of organic semiconductors (OSCs) as active materials in electronic devices, due to the possibility to process these materials at low temperature on flexible substrates, fabricate them on large-area, and upscale their fabrication using cost-effective strategies such as printing. Because of these features, organic electronic devices are rapidly emerging as biosensors for biomarkers, with a high potential for becoming a high-throughput tool even deployable at the point-of-care.

One of the most used and studied platforms is the organic electrochemical transistor (OECT). OECTs have been largely used as biosensors in order to transduce and amplify electrical signals or detect biological analytes upon proper functionalization with specific biorecognition units. OECTs can operate at low voltages, are easy to fabricate on different substrates, and are compatible with the aqueous environment, and can therefore be interfaced with living systems, ranging from mammals to plants. The OECT device configuration includes a gate electrode that modulates the current in the channel through an electrolyte, which can be not only a buffered solution but even a complex biological fluid. When OECTs are operated as biosensors, the sensing mechanism relies on the current variation generated from specific reactions with the analyte of interest. These devices are paving the way to the development of point-of-care technologies and portable biosensors with fast and label-free detection. Moreover, OECTs can help to reveal new biological insight and allow a better understanding of physiological processes.

During my PhD, I focused on design, fabrication, and validation of different OECT-based biosensors for the detection of biomarkers that are relevant for healthcare applications, thus showing their high potential as a proper sensing platform. We developed sensors towards different analytes, ranging from small molecules to proteins, with ad hoc designed materials strategies to endow the device with selectivity towards the species of interest. Most notably, I also demonstrated the possibility of integrating OECTs in plants, as an example of interfacing these biosensors with living systems. In the first two papers, we developed screen printed

OECTs, presenting PEDOT:PSS as the semiconducting material on the channel. In the first case, the device also featured a PEDOT:PSS gate electrode which was further functionalized with biocompatible gelatin and the enzyme urease to ensure selectivity toward the analyte of interest, namely urea. The biosensor was able to monitor increasing urea concentrations with a limit of detection of 1 μM . In the second paper the screen-printed carbon gate electrode was first modified with platinum and then we ensured selectivity towards the analyte uric acid, a relevant biomarker for wound infection, by entrapping urate oxidase in a dual-ionic-layer hydrogel membrane to filter out charged interfering agents. The biosensor exhibited a 4.5 μM limit of detection and selectivity even in artificial wound exudate. In the third paper we designed an interleukin-6 (IL6) OECT based biosensor able to detect the cytokine down to the pM regime in PBS buffer. The mechanism of detection relied on the specific binding between an aptamer, used as sensing unit on the gate electrode, and the IL6 in solution, allowing for detection ranging from physiological to pathological levels.

In the last two papers we developed OECT based biosensors to be interfaced with the plant world. In the fourth paper we presented a glucose sensor, based on the enzyme glucose oxidase (GOx) to detect glucose export from chloroplasts. In particular, we demonstrated real-time glucose monitoring with temporal resolution of 1 minute in complex media. In the fifth paper, we developed implantable OECT-based sugar sensors for *in vivo* real-time monitoring of sugar transport in poplar trees. The biosensors presented a multienzyme-functionalized gate endowing the device with specificity towards glucose and sucrose. Most notably, the OECT sensors did not cause a significant wound response in the plant, allowing us to demonstrate that OECT-based sensors are attractive tools for studying transport kinetics in plants, *in vivo* and real-time.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Oorganiska material har varit huvudaktörerna i halvledarindustrin under de senaste fyrtio åren. Det har emellertid funnits ett kontinuerligt intresse och tillväxt inom forskning för tillämpningar av organiska halvledare (OSC) som aktiva material i elektroniska apparater, på grund av möjligheten att bearbeta dessa material vid låga temperaturer, på flexibla substrat, tillverka dem på stora ytor och skala med kostnadseffektiva strategier såsom tryckning. På grund av dessa funktioner växer organiska elektroniska enheter snabbt fram som biosensorer för biomarkörer, med hög potential att bli ett genomgående verktyg som även kan användas vid vårdplatsen. En av de mest använda och studerade plattformarna är den organiska elektrokemiska transistorn (OECT). OECT har i stor utsträckning använts som biosensorer för att transducera och förstärka elektriska signaler eller upptäcka biologiska analyter vid korrekt funktionalisering med specifika bio-igenkännande enheter. OECT kan fungera vid låga spänningar, de är lätta att tillverka på olika substrat och kompatibla med den vattenhaltiga miljön, därför kan de kopplas till levande system, allt från däggdjur till växter. Dessa enheter banar väg för utvecklingen av point-of-care teknologier och portabla biosensorer med snabb och etikettfri detektering. Dessutom kan de hjälpa till att avslöja nya biologiska insikter och möjliggöra en bättre förståelse för fysiologiska processer.

OECT-enhetens konfiguration inkluderar en gate-elektrod som modulerar strömmen i kanalen genom en elektrolyt, som inte endast behöver vara en buffrad lösning utan till och med en komplex biologisk vätska. När OECT används som biosensorer är avkänningsmekanismen beroende av spänningsvariationen som genereras från specifika reaktioner med analyten av intresse. Under min doktorsexamen fokuserade jag på design, tillverkning och validering av olika OECT-baserade biosensorer för detektering av biomarkörer som är relevanta för tillämpningar inom vård och visar därmed deras höga potential som riktiga avkänningsplattformar. Vi utvecklade sensorer för olika analyter, allt från små molekyler till proteiner, med ad hoc-designad materialstrategi för att ge enheten selektivitet gentemot arten av intresse. Framför allt visade jag också möjligheten att integrera OECT i växter, som ett exempel på gränssnittet mellan dessa biosensorer och levande system. I de två första artiklarna utvecklade vi skärmtryckta OECT:er och presenterade PEDOT:PSS som halvledande material i kanalen.

I det första fallet innehöll anordningen också en PEDOT:PSS gate-elektrod som ytterligare funktionaliserades med biokompatibelt gelatin och enzymet ureas för att säkerställa selektivitet mot analyten av intresse, nämligen urea. Biosensorn kunde övervaka ökande ureakoncentrationer med en detektionsgräns på 1 μM . I den andra artikeln modifierades först den skärntryckta kol baserade gate-elektroden med platina och sedan säkerställde vi selektivitet mot analyten urinsyra, en relevant biomarkör för sårinfektion, genom att fånga upp uratoxidas i ett dubbelt jonskikt hydrogel-membran för att filtrera bort laddade störande medel. Biosensorn uppvisade en detektions- och selektivitetsgräns på 4.5 μM även i artificiell sårutsöndring. I den tredje artikeln designade vi en interleukin-6 (IL6) OECT-baserad biosensor som kunde detektera cytokinnivåer ner till pikomolar nivåer i PBS-buffert. Detektionsmekanismen är beroende av den specifika bindningen mellan en aptamer, som används som avkänningsenhet hos gate-elektroden, och IL6 i lösning, vilket möjliggör detektering som sträcker sig från fysiologiska till patologiska nivåer.

I de två sista artiklarna utvecklade vi OECT-baserade biosensorer för att koppla samman med växtvärlden. I den fjärde artikeln uppvisade vi en glukossensor, baserad på enzymet glukosoxidas (GOx) för att detektera glukosexport från kloroplast. I synnerhet visade vi glukosövervakning i realtid med tidsmässig upplösning på 1 minut i komplexa medier. I den andra artikeln utvecklade vi implanterbara OECT-baserade sockersensorer för in vivo, realtidsövervakning av sockertransport i poppelträd. Biosensorerna presenterade en multienzymfunktionaliserad gate som ger enheten specificitet mot glukos och sackaros. Framför allt orsakade inte OECT-sensorerna signifikant sårrespons i växterna, vilket gjorde att vi kunde visa att OECT-baserade sensorer är attraktiva verktyg för att studera transportkinetik i växter, in vivo och realtid.

SOMMARIO

I materiali inorganici sono stati i protagonisti dell'industria dei semiconduttori negli ultimi quarant'anni. Tuttavia, c'è un continuo interesse e crescita nella ricerca e nell'applicazione di semiconduttori organici (OSC) come materiali per dispositivi elettronici, grazie alla possibilità di processarli a bassa temperatura, su substrati flessibili e di fabbricarli utilizzando diverse strategie come lo stampaggio. Grazie a queste caratteristiche, i dispositivi elettronici organici stanno rapidamente emergendo come piattaforme per biosensori di biomarcatori, con un forte potenziale di diventare uno strumento utilizzabile come point-of-care.

Una delle piattaforme più utilizzate e studiate è il Transistor Elettrochimico Organico (OECT). Gli OECT sono stati ampiamente utilizzati come biosensori per trasdurre e amplificare segnali elettrici o rilevare analiti biologici previa funzionalizzazione con specifiche unità di bioriconoscimento. Gli OECT possono operare a basso voltaggio, sono facili da fabbricare su diversi substrati e compatibili con l'ambiente acquoso, quindi possono essere interfacciati con sistemi viventi, dai mammiferi al mondo vegetale. La configurazione degli OECT include un elettrodo di gate che modula la corrente nel canale attraverso un elettrolita, che può essere non solo una soluzione tampone ma anche un fluido biologico complesso. Quando gli OECT vengono utilizzati come biosensori, il meccanismo di rilevamento si basa sulla variazione di corrente generata da reazioni specifiche con l'analita di interesse.

Durante il mio dottorato di ricerca, mi sono concentrata sulla progettazione, fabbricazione e validazione di biosensori basati su OECT per il rilevamento di biomarcatori per applicazioni cliniche e ambientali, mostrando il loro potenziale come piattaforma di rilevamento. Abbiamo sviluppato sensori verso diversi tipi di analiti, dalle piccole molecole alle proteine, usando materiali progettati ad hoc per dotare i dispositivi di selettività verso le specie di interesse. In particolare, abbiamo anche dimostrato la possibilità di integrare gli OECT nelle piante, per dimostrare la loro compatibilità con sistemi viventi. Nei primi due articoli, abbiamo sviluppato OECT stampati, che presentano PEDOT:PSS come materiale semiconduttore sul canale. Nel primo caso, il dispositivo presenta anche un elettrodo di gate di PEDOT:PSS che è stato ulteriormente funzionalizzato con gelatina biocompatibile e l'enzima ureasi per garantire la selettività verso l'analita di interesse, ovvero l'urea. Il biosensore è in grado di monitorare varie concentrazioni di urea con un limite di detection di $1\mu\text{M}$. Nel secondo articolo l'elettrodo

di gate di carbonio stampato è stato prima modificato con platino e successivamente abbiamo assicurato la selettività del biosensore verso l'acido urico, un biomarcatore rilevante per l'infezione di ferite, intrappolando l'urato ossidasi in una membrana a doppio strato ionico per filtrare gli interferenti. Il biosensore ha un limite di rilevamento di 4,5 μM anche in essudato artificiale ed è selettivo per l'analita di interesse. Nel terzo articolo abbiamo progettato un biosensore per la detection di interleuchina-6 (IL6) in grado di rilevare livelli di citochina in concentrazioni pM in PBS. Il meccanismo di rilevamento si basa sul legame specifico tra un aptamero, utilizzato come unità di rilevamento sull'elettrodo di gate, e l'IL6 in soluzione, consentendo il rilevamento da livelli fisiologici a patologici.

Negli ultimi due articoli abbiamo sviluppato biosensori basati su OECT per interfacciare il mondo vegetale. Nel quarto articolo abbiamo presentato un sensore, basato sull'enzima glucosio ossidasi (GOx) per rilevare l'esportazione di glucosio da cloroplasti. In particolare, abbiamo dimostrato il monitoraggio di glucosio in tempo reale con una risoluzione temporale di 1 minuto. Nel secondo articolo, abbiamo sviluppato sensori impiantabili basati su OECT per il monitoraggio in vivo e in tempo reale del trasporto di zuccheri negli alberi di pioppo. I biosensori presentano un elettrodo di gate funzionalizzato con diversi enzimi in modo da conferire al dispositivo una specificità nei confronti di glucosio e saccarosio. In particolare, i dispositivi non hanno causato singificativi danni al tessuto, consentendoci di dimostrare che i sensori basati su OECT possono rappresentare soluzioni alternative per studiare la cinetica di trasporto nelle piante, in vivo e in tempo reale.

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LIST OF PAPERS AND CONTRIBUTION

Paper I

Label free urea biosensor based on organic electrochemical transistors.

Marcello Berto, **Chiara Diacci**, Lorenz Theuer, Michele Di Lauro, Daniel T Simon, Magnus Berggren, Fabio Biscarini, Valerio Beni, Carlo A Bortolotti.

Flexible and Printed Electronics, 2018.

Contribution: device printing, device characterization

Paper II

Flexible Printed Organic Electrochemical Transistors for the Detection of Uric Acid in Artificial Wound Exudate.

Marina Galliani, **Chiara Diacci**, Marcello Berto, Matteo Sensi, Valerio Beni, Magnus Berggren, Marco Borsari, Daniel T Simon, Fabio Biscarini, Carlo A Bortolotti.

Advanced Materials Interfaces, 2020.

Contribution: experimental and device design, master student experiment supervision, manuscript writing.

Paper III

Organic electrochemical transistor-based aptasensor for interleukin-6 label-free detection.

Chiara Diacci, Marcello Berto, Tero Petri Ruoko, Samuel Lienemann, Daniel T. Simon, Magnus Berggren, Marco Borsari, Fabio Biscarini, Carlo A. Bortolotti.

Manuscript in preparation

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

Paper IV

Real-Time Monitoring of Glucose Export from Isolated Chloroplasts Using an Organic Electrochemical Transistor.

Chiara Diacci, Jee Woong Lee, Per Janson, Gwennaël Dufil, Gábor Méhes, Magnus Berggren, Daniel T Simon, Eleni Stavrinidou.

Advanced Materials Technologies, 2020.

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

Paper V

Diurnal in vivo xylem sap glucose and sucrose monitoring using implantable organic electrochemical transistor sensors.

Chiara Diacci, Tayebbeh Abedi, Jee Woong Lee, Erik O Gabrielsson, Magnus Berggren, Daniel T Simon, Totte Niittylä, Eleni Stavrinidou.

iScience, 2021.

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

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INTRODUCTION

Biosensor

Biosensors are systems/devices able to translate a biological event into a readout, that is proportional to the concentration of a given analyte in a sample. Nowadays, biosensors are essential tools present in various fields and are employed for many applications, from clinical tests to environmental monitoring. Typically, a biosensor is described by the following elements (fig. 1): i) the input, which is the analyte to be detected (e.g., metabolites, proteins, DNAs); ii) the biorecognition element, which is the biomolecule able to recognise specifically the analyte of interest (e.g., antibody, DNA, enzyme, cell); iii) the transducer, which allows the conversion of the physical/chemical event between input and biorecognition element into a measurable signal (the main transducers being optical, electrochemical, magnetic and amperometric); iv) the output, which is the processed signal (the output can be displayed under the form of colour, current or voltage variation).

When designed, a biosensor should feature some characteristics and attributes: it should be selective, being able to detect a specific analyte in a complex sample which may contains interfering molecules. Its response should be reproducible, i.e. yielding same the signal (within a given, quantifiable tolerance) for the same analyte concentration, in different experiments, and it should be sensitive to the analyte, in a concentration range useful for the diagnosis¹. Depending on the transducing principle used to detect the biological event in the sensing platform, biosensors can be divided in:

- Optical biosensors: these transducers are exploiting the optical properties of the light, taking advantage of phase or intensity changes taking place when the analyte binds the biorecognition element². The most established biosensors that are based on optical transducer are Surface Plasmon Resonance³ (SPR), Localized Surface Plasmon Resonance⁴ (LSPR) and Surface-enhanced Raman Scattering⁵ (SERS), which represent label-free techniques (no incubation with external molecules). While other optical biosensor, such as fluorometric and colorimetric assay⁶ are based on the addition of molecules to reveal optically the biding between analyte and sensing unit.

- Electrochemical biosensors: these devices exploit electrochemical processes to detect an analyte in solution. They measure variations of electrical signals caused by the binding

between analyte and biorecognition element. Commonly the event is displayed as variation of current, potential or impedance. The most used techniques include Cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS)⁷.

- Magnetic biosensors: these devices employ the unique properties of magnetic/paramagnetic particles to monitor the biological interaction between analyte and sensing unit. They detect changes in magnetic properties upon molecules interaction⁸.

- Piezoelectric biosensors: these devices exploit piezoelectric crystal to detect biological binding events. They measure changes in the oscillation of the piezo, that are caused by mass changes upon binding between biorecognition unit and analyte⁹.

Figure 1: Building blocks of a biosensor. The input is the analyte to be detected and it can be represented by metabolites, proteins, DNAs, and action potentials. The biorecognition element is crucial to detect specifically the analyte of interest: it can be an antibody, a DNA/RNA oligo, an enzyme or even a whole cell. The transducer translates the biological signal into a measurable signal, which can be amperometric, potentiometric or optical. The output displays the signal, and can be under the form of current, voltage or light.

The first biosensor was introduced in 1956, by the efforts of Leland C. Clark Jr, who used a platinum electrode vs an Ag/AgCl reference electrode, to detect different oxygen concentrations in solution ¹⁰ (fig. 2). This first demonstration led to the development of biosensors that were exploiting enzymatic units to detect glucose¹⁰ (by Leland Clark in 1962) and urea¹¹ (by Guilbault and Montalvo Jr in 1969). In 1980, the first immunosensor was proposed¹², followed by the first Surface Plasmon Resonance (SPR)-based immunosensor in 1983¹³ and its commercialization by Biacore in 1990¹⁴. Biosensor innovation moved forward

with the creation of DNA based sensors¹⁵, able to recognize complementary oligonucleotide strands and thus opening the way to genetic tests, and with the introduction of nanomaterials to enhance the biosensor performance^{16,17}. Nowadays, biosensors are not necessarily confined to the laboratory bench and are ready to be interfaced directly with biological systems, under the form of wearable^{18,19} or implantable sensors^{20–22}.

Figure 2: Timeline for biosensor development.

Biosensor in healthcare and agriculture

Biosensors are crucial tools in the healthcare system but also in many other fields such as food control, agriculture and environmental monitoring. In medical care, biosensors, together with other clinical tests, are used for diagnosis, screening and monitoring to evaluate pathological processes, thus representing an imperative necessity in the healthcare. Nowadays the healthcare system has an enormous impact on our economy, and just in the USA it is exceeding the 2.7\$ trillion per year²³. The cost of the diagnostic tests takes 10% of the budget and it is rapidly rising over time, with the introduction of molecular diagnostic for personalised medicine. The demands for screening and diagnosis are increasing and becoming virtually infinite, but the resources in terms of laboratory equipment and specialized handwork are finite, thus creating an urgent, critical situation. Equally important and challenging is the use of biosensors for environmental and agricultural applications²⁴. Climatic changes are becoming less predictable and more disruptive, causing huge economic problems in

agriculture and ecological disasters. Thus, in view of establishing sustainable agriculture systems, biosensors would be highly necessary for smart monitoring, enabling control of plant physiology in real time and providing support to take environmentally affordable decisions²⁵.

For these reasons, there is a clear necessity to introduce disposable and cost-effective biosensors to advance healthcare and agriculture. Up to date, among the most used and standardized tests for analyte detection and quantification are Enzyme Linked Immunosorbent Assay²⁶ (ELISA), Mass Spectrometry²⁷ or High Performance Liquid Chromatography²⁸ (HPLC). Those techniques exhibit high sensitivity and selectivity towards a very large portfolio of analytes, even in complex fluids. In addition, they are highly reliable and reproducible, an essential characteristic for clinical diagnosis. However, these methods require sample processing, thus increasing the amount of sample that needs to be collected, specialized personnel and equipment and are typically time consuming. In order to solve these limitations, research is focusing its efforts in automating biosensors, transforming them in a competitive technology that will abandon the laboratory bench in favour of portable devices embedded in an easy, user-centred set-up. To face the high demand and the new requirements biosensors should be compatible for large scale production, fabricated with cost-effective materials, reliable and robust. New biosensing platforms for advanced detection devices should be miniaturized systems, implantable or in field deployable for point-of-care applications in home testing. The introduction of new nanomaterials, such as carbon nanotubes²⁹ (CNTs) or nanoparticles³⁰ opened to many new possibilities for the biosensing field, together with new techniques for biosensor fabrication on conformable and ultra-thin substrates, such as parylene^{31–33}. Carbon-based, 2D materials are not the only novel players in the field of biosensing. Organic polymers are also among the new materials used for advancing the technology and are playing a major role in biosensor development, introducing innovative fabrication methods and new detection properties.

Aim of the thesis

The aim of this thesis is to develop organic based biosensors for the detection of various biomarkers. The fabrication of these devices includes cost-effective techniques for the creation of disposable and portable devices together with traditional microfabrication methods for creating ad hoc biosensors for plant interfacing. In this thesis I have focused on

understanding the working principle of Organic Electrochemical Transistors (OECTs) and their properties for using them, after functionalization as effective biosensing platforms.

ORGANIC ELECTRONICS

Inorganic materials have been the main protagonists of the semiconductor industry for the past forty years. Traditional silicon-based electronics overcame many challenges in term of fabrication and exponentially increased demand, but they still present some limitations³⁴. For these reasons, there has been a growing interest in the application of organic semiconductors (OSCs) as active materials in electronic devices. OSCs include different types of materials, from small molecules to polymers and nanotubes, each of which can exist in the form of molecular crystals or amorphous films³⁵. OSCs are materials that present carbon atoms in their backbone and π -conjugated orbitals which contain delocalised electrons. These π orbitals are defined as the highest occupied molecular orbital (HOMO) and take the role of the valence band for inorganic semiconductors, while π^* orbitals are defined as lowest unoccupied molecular orbital (LUMO) and take the role of conduction band. Because of the π conjugated system the energy gap between LUMO and HOMO is small enough to permit charge transfer (fig. 3).

Figure 3: Structure and energetic levels of molecular orbitals for unconjugated and conjugated organic polymers. The presence of double bounds in organic conjugated polymers reduce the energy gap between HOMO and LUMO orbitals, allowing charge transfer from the π band to the π^* band.

When an electron jumps from HOMO to LUMO it causes a distortion of electron spatial distribution, causing a geometrical distortion and defining the region carrying the charge as a polaron. For some conductive polymers (e.g., polythiophene, polypyrrole) it is possible to

identify a second structure with different energy, called bipolaron, which represent the bication state of the polymer³⁶. Charge transport properties in OSCs depend on the molecular packing, the energy gap between HOMO and LUMO, the temperature, and the degree of order in the solid state³⁷. Pure semiconducting polymers, without any addition/subtraction of external charges, show very poor electrical conductivity (insulator). Conductivity can be increased by adding dopant molecules that can donate/accept electrons and define which primary charge carriers are transported in the semiconducting polymer. The delocalisation of π orbitals provides molecular stability giving low ionisation potential and high electron affinity, facilitating the doping process of the organic conjugated polymer. Organic semiconductors can be classified as: **p-type** (transporting holes) or **n-type** (transporting electrons). The difference lays in the primary charge carrier. In n-type semiconductors, an electron jumps from the HOMO level of the dopant to the LUMO of the semiconductor, allowing charges (electrons) to move through LUMO orbitals. In p-type semiconductors charge transport is described by hopping (jumping from one molecule to another), because an electron is taken from the HOMO orbital of the semiconductor and added to the LUMO orbital of the dopant molecule, leaving a hole in the conductive polymer backbone³⁸ (fig. 4).

Figure 4: Schematic of energetic levels for dopant molecules. For p-type doping, an electron jumps from the HOMO orbital of the organic material to the LUMO orbital of the dopant molecule, leaving a hole as charge carrier in the conductive polymer. For the n-type doping an electron jumps from the HOMO orbital of the dopant molecule to the LUMO orbital of the organic material, adding an electron as charge carrier in the conduction band.

Organic Bioelectronics

Bioelectronics was first introduced by Luigi Galvani in the 18th century, when during the study of biological electrical signals, he connected a frog leg to its spinal cord, making it twitch³⁹.

Since then, the applications of electronic devices for interfacing the biological world have grown exponentially, resulting in the creation of biosensors, electronic implants (e.g., pacemaker, cochlear implant) and drug delivery devices^{40–42}. The main limitation of traditional bioelectronic devices is represented by the material used to communicate with living systems. Organic conductive polymers and molecules represent ideal materials for interfacing biology and overcoming the limitations of inorganic semiconductors. The main advantage lies in the property of OSCs to conduct both electronic and ionic charges^{43,44}; ions in fact, are one of the main signalling molecules that drive biological processes. Moreover, organic conductive materials, in contrast with silicon-based electronics, can interface directly with the biological milieu, because of their oxide-free surfaces, allowing a deeper interaction with events happening in biological fluids. The use of OSCs enables the fabrication of more biocompatible devices; organic materials are solution processable and allow easy chemical modifications, permitting the creation of soft or flexible devices that decrease the rheological mismatch between the biological tissue and the electronic component. Some organic polymers present a porous configuration, which can unlock specific properties and enhance neural interfacing devices. This 3D structure permits the storage of high number of charges at the electrode interface, decreasing the impedance and improving the signal to noise ratio. One of the most used and studied conductive polymers for bioelectronic devices is poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) (fig. 5). This material is a polymeric mixed ion-electron conductor characterised by high hole conductivity ($>1000 \text{ S cm}^{-1}$), chemical stability, and versatility in manufacturing processes. PEDOT:PSS comprises rich polyanion (PSS⁻) regions interfacing a few PEDOT domains (fig. 5), resulting in a hygroscopic material often presenting a porous gel structure⁴⁵. The hole density in PEDOT:PSS is modulated by ions in the electrolyte, entering the polymeric structure by application of a potential vs a reference electrode, creating a volumetric ion storage capacitance dependent on film thickness⁴⁶. PEDOT:PSS is commercially available and because of its solution processability is compatible with a wide range of fabrication techniques, including electropolymerization, spin-coating, printing, and physical vapour deposition (PVD). PEDOT based devices have been successfully employed in bioelectronics applications as electrode arrays to record brain signals⁴⁷, as cell scaffolds to stimulate and measure cell growth^{48–51}, as biosensors to record biomarkers^{22,52–54}, and as active materials for drug delivery devices⁵⁵.

Figure 5: a) Chemical structure of the PEDOT:PSS conductive polymer. b) Representation of PEDOT:PSS film on a substrate. The magnification shows the peculiar porous polymeric structure, which contain rich PSS clusters (grey), interfaced by fewer PEDOT regions (blue).

Fabrication methods

The application of organic molecules and polymers in electronics has experienced a tremendous growth. This is because OSCs exhibit several practical advantages compared to traditional inorganic semiconductors. Organic polymers have low melting point and high solubility; characteristics which unlock the possibility to use these materials at low temperature and with solution processable techniques on flexible substrates (e.g., plastic or paper). OSCs are compatible with innovative and versatile fabrication techniques, such as solution casting, spin coating, ink-jet printing, and screen-printing, enabling fabrication on large-area and with cost-effectiveness^{56,57}. Nevertheless, organic polymers are compatible with traditional photolithographic techniques, such as vacuum evaporation, to achieve miniaturised systems for more challenging targets.

Printing techniques, commonly used in the graphic arts, can be applied to soluble organic conductive materials. The screen-printing process involves pushing the ink (organic polymer in solution) through a mesh that has openings on top, representing the pattern. In more details, the screen is covered by the ink, and a squeegee presses it through the mesh, transferring the pattern to the substrate. A well-defined pattern in screen-printing is often obtained using high viscosity ink solution, in order to produce thick film ($>1\ \mu\text{m}$), while the resolution is determined from the mesh size and is generally in the range of $100\ \mu\text{m}$ ⁵⁶. One of the most peculiar advantages of using organic semiconducting molecules to fabricate electronic circuit and devices is represented by the fact that some of these molecules can actively interact with biological units, polymerising inside the living system itself^{58–60}. Recently

it has been proved that 3,4-ethylenedioxythiophene (EDOT) based trimer can be absorbed by biological system and be polymerised by enzymatic units, physiologically present in the tissue, to create conductive pathways within the biological system⁶¹.

ORGANIC TRANSISTORS

Transistor

Transistors are three terminal devices able to amplify and/or switch electronic signals. All transistors share the same basic structure composed of input/output electrodes in contact with the semiconducting material in the channel and a control electrode contacting the semiconductor through a dielectric medium, that modulates the current flow. Nowadays transistors are essential components and building blocks for electrical circuits and are present in every ICU (integrated circuit unit). The first transistor was developed in 1947 at Bells Lab by the physicists John Bardeen, Walter Brattain, and William Shockley, whose resulted in a Nobel prize in 1956⁶². In their first prototype (fig. 6a) they developed a point-contact transistor (bipolar junction transistor) using germanium as semiconducting material on a metal base and two gold contacts on a triangular support to contact the channel. A more recent alternative to the bipolar junction device is the field effect transistor (FET). FET geometry includes the familiar three electrodes: source and drain electrodes in contact with the semiconductor, and a gate electrode separated by an insulator to modulate the current in the channel (fig. 6b).

Figure 6: a) Reproduction of the first transistor, based on germanium as semiconducting material, made in 1947 by Jhon Bardeen, Walter Brattain and William Shockley. b) Schematic of a Field Effect Transistor (FET), presenting Source and Drain electrode in contact with the semiconductor and the Gate electrode on top of the insulating material.

FET devices didn't successfully perform until 1957, when the engineer Mohamed Atalla, studying the semiconducting properties of silicon, had the brilliant idea to passivate the surface with a layer of silicon oxide⁶³. This step overcame the problem related to the surface barrier that was preventing the electric field to influence the active material and thus

introduced the metal-oxide-semiconductor field-effect transistor (MOSFET). MOSFETs were also the first miniaturised transistors compatible with a production process that was suitable for large scale, opening the way to high density integrated circuits. Transistors can be used for various purposes including logic gates in digital circuits or as amplifiers. Since the aim of the thesis is to take advantage of the transistor amplification property, we will not go deeper into their logic switching mechanisms.

Organic based transistors

Organic transistors share the same working principle as their inorganic counterparts. They are three electrode devices, in which the semiconducting material is represented by an organic polymer or molecule. The source and drain electrodes are connected to each other through a layer of OSC, while the gate electrode couples to the OSC through a dielectric which can be solid or liquid. In the case of an organic field effect transistor (OFET), the gate is separated from the channel by a thin layer of dielectric material (fig. 7a). The charge accumulation and current modulation in the channel is driven by the field effect, generated in the insulating material by the applied voltage at the gate electrode⁶⁴.

In some cases organic transistors operate with an electrolyte as the dielectric between gate and OSC, exploiting the mobile ions as charges to induce a field effect. In this configuration the gate can modulate the current in the channel through an electrolyte, which not only can be represented by a buffer solution, but also by complex biological fluids. The ion interaction with the OSC defines two different kinds of organic transistors: the electrolyte-gated field effect transistor (EGOFET)⁶⁵ and the organic electrochemical transistor (OECT)⁶⁶.

Figure 7: Schematic representation of organic transistors. a) OFET geometry presenting a solid dielectric material (blue) separating the organic polymer in the channel and the top gate electrode. b) EGOFET geometry presenting a solution as dielectric to separate the gate electrode and the conducting polymer. Upon bias application, ions migrate at the interface semiconductor/electrolyte, creating an electrical double layer. c) OECT geometry presenting a solution as dielectric to separate the gate electrode and the conducting polymer. In contrast to EGOFET, upon bias application, ions migrate inside the bulk of the conducting polymer.

The first demonstration of an EGOFET (water-gated) was reported by Kergoat *et al.* in 2010 using rubrene and poly(3-hexylthiophene) (P3HT) as organic materials⁶⁷. More generally, EGOFETs present an organic semiconductor in the channel which is impermeable to ions (fig. 7b). Therefore a voltage applied between gate and source (V_{GS}) induces the formation of electrical double layers (EDLs) at the gate/electrolyte and semiconductor/electrolyte interfaces. This process will dope/dedope the semiconducting polymer in the channel, modulating charges in proximity of the EDL, and thus modulating the current between source and drain (I_{DS}). The EDL is composed by the compact Helmholtz layer, made by a single row of solvated ions and a diffuse layer, and it can be compared to a capacitor⁶⁵. Because the capacitance is inversely proportional to the thickness and the double layer is extremely small, EGOFETs display very high capacitance, therefore they have the possibility to be operated at potentials below 1 V, showing significant advantages such as biological compatibility⁶⁸ and low-power consumption⁶⁵.

The OECT, in contrast with the EGOFET, presents a permeable polymer as semiconducting material in the channel. A voltage applied between source and gate drives the ions from the electrolyte inside the polymer matrix, consequently modulating the current in the channel (fig. 7c). The first OECT was described in 1980 by Wrighton *et al.*⁶⁹ using polypyrrole as OSC, but its popularity increased after some years when Andersson and Nilsson *et al.*⁷⁰ fabricated

a device using PEDOT:PSS as active material. Up till now researchers have mainly exploited PEDOT:PSS as semiconducting polymer for many applications, but also focused their efforts in creating new geometries and finding new materials to optimise OECTs. As the name “electrochemical” suggests, the device operation is supposed to rely on Faradaic processes, involving reduction and oxidation of the conductive polymer. However, “electrochemical” may not be the best name, since some models describe the function as bulk capacitance rather than electrochemical changes of the channel material⁷¹. As recently proposed for a PEDOT:PSS based electrode, conductive polymers present a volumetric capacitance^{46,72}. PEDOT:PSS’s unique structure can be described by rich PSS regions carrying the fixed anions, and fewer PEDOT chains carrying the holes (fig. 5b). Upon bias application cations from the electrolyte are injected into the bulk of the polymer, and holes are extracted through the metal in contact with the polymer, decreasing their density. A capacitor is formed at the interface between the fixed sulfate moieties on the PSS chain and the injected cations, which present the same capacitance thickness described by the Helmholtz model in EGOFETs. Since the whole polymer contains many of these “sites” which are giving rise to capacitors, we can consider for some conductive polymer a capacitance which depends on the volume of the film⁷³. OECT can be based on p-type conducting polymers, such as PEDOT:PSS, or more challenging n-type conducting polymers, e.g., poly(benzimidazobenzophenanthroline) (BBL)⁷⁴ or NDI-T2⁷⁵. PEDOT:PSS is a p-type doped conductive polymer which presents mobile holes on the PEDOT backbone. The holes, compensated by the PSS fixed ionic charges, create a current flow in the channel upon bias application between source and drain. OECTs based on p-type polymers work in depletion mode, meaning that without gate bias the current flows in the channel, creating an ON state. Upon positive gate bias application, cations in the electrolyte are injected into the polymeric matrix, compensating the fixed anions (PSS) and de-doping the channel. The decrease of compensating charges leads to a decrease of the number of holes and lower current values⁷⁶. On the contrary, n-type polymer based OECTs work in accumulation mode. Without gate bias there is no/limited current flow, creating an OFF state. Upon bias application ions are injected in the channel, accumulating opposite charges, which are responsible for the current when a voltage is applied between source and drain⁷⁶.

OECT device physics

The Bernards and Malliaras model⁷⁷ describes the OECT working mechanism dividing it into two circuits (fig. 8): (i) an electronic circuit, composed of a channel resistance, dependent on the voltages applied at the gate, and (ii) an ionic circuit, which describes electrical components in the solution. The channel resistance (R_{ch}) in the electronic circuit describes the charge flow between source and drain in accordance with Ohm's law and it varies with the charge amount accumulated into the porous channel. The ionic circuit is composed of the resistance of the electrolyte (R_e), representing ion movement, connected in series with two capacitances describing the ion storage at the gate (C_g) and in the channel (C_{ch}).

Figure 8: Schematic of an Organic Electrochemical Transistor (OECT) presenting a solution as dielectric material and a top gate electrode immersed into the electrolyte. The magnifications show the ionic circuit, composed by the gate and the channel capacitance in series with the electrolyte resistance and the electronic circuit of the channel, composed by a variable resistance.

OECTs can be mathematically described, relying on their similarity with MOSFETs. The current in the channel in the steady behaviour (not time dependent) can be described for two regimes: the linear regime (when V_{DS} is higher than $V_{GS} - V_T$) and the saturation regime (when V_{DS} is lower than $V_{GS} - V_T$).

$$I_{DS} = \mu C^* \frac{Wd}{L} \left(1 - \frac{V_{GS} - V_T}{V_T} \right) V_{DS}; \text{ linear regime} \quad (1)$$

$$I_{DS} = -\mu C^* \frac{Wd}{L} \frac{(V_{GS} - V_T)^2}{2V_T}; \text{ saturation regime} \quad (2)$$

Where I_{DS} is the current in the channel, μ is the charge mobility in the semiconductor, and C^* the volumetric capacitance. W , d , and L are the geometrical dimensions width, thickness, and length of the channel, respectively. V_{GS} is the gate voltage, V_{DS} the drain voltage, and V_T the threshold voltage, which is the voltage at which the device is in off-state, and depends on capacitance and the intrinsic doping level of the polymer. The transconductance (g_m) is an important parameter to address in order to optimise an OEET and it is described by the equation:

$$g_m = \mu C^* \frac{Wd}{L} (V_T - V_{GS}) \quad (3)$$

The transconductance can be calculated from Eqns. 1 and 2 and is the derivative of the current in the channel with respect to the gate voltage. In OEETs, g_m scales with the channel dimension, and specifically with the thickness, in contrast with OFETs. This behaviour arises from the ionic charges penetrating the bulk of the polymer and not accumulating at the interface. Rivany *et al.* also demonstrated that g_m can be modulated (for fixed thickness) by increasing W/L ⁷⁸. Other parameters studied to optimise OEET devices are charge mobility μ and capacitance C^* , which are intrinsic to the semiconducting material⁷⁹.

OEETs have been fabricated using different geometries. In the bottom-contact configuration (BC), the conducting polymer is deposited on top of the substrate and S and D electrodes while the gate is separated from the channel by the electrolyte. In the top-contact (TC) configuration, the S and D electrodes are located on top of the channel, formed by the semiconducting polymer, while the gate electrode is in the same position as for the BC configuration. A vertical geometry for OEETs, similar to the OFET configuration, has been recently developed⁸⁰. The vertical position of the electrodes allows deposition of a thin layer of semiconducting polymer, reducing the length between source and drain and thus increasing transconductance. This type of OEET is mostly used for in vivo interfaces because of their smaller size and high signal amplification.

OEET as biosensor

OEETs offers a unique platform and an attractive tool for biosensing applications. The volumetric capacitance, arising from the ion penetration into the bulk of the polymeric channel, allows high signal amplification, and thus operation in low voltages regimes,

preventing electrolysis and permitting low power consumption⁸¹. Moreover, OECTs exhibit stability in aqueous environments, including body fluids, and allow easy functionalization strategies to interface living system, making them an appealing candidate for in vitro and in vivo applications⁸². OECT fabrication methods offer the possibility to fabricate them with low-cost printing techniques^{83,84} or with photolithography for more challenging applications^{22,32,33,85}. Moreover, they can be integrated with microfluidics⁸⁶, paving the way for lab-on-chip technologies. OECTs have been used for a broad range of biological applications, to record electrophysiological signals (e.g. electrocardiogram^{87,88} and electroencephalogram³²), as ion sensors^{89,90}, as enzymatic sensors^{54,86,91–93}, and immunosensors^{94–96}. OECTs as biosensors can be operated exploiting non-faradic or faradic reactions. The former is based on capacitive changes of the EDL at the gate electrode or at the channel that results in a minor/major ion electrostatic compensation and consequently a measurable current variation^{48,72}. On the contrary, in faradic processes, there is the presence of electrochemical reactions between the analyte in solution and the system. These reactions are called reduction (losing an electron) or oxidation (gaining an electron) and are involving electroactive molecules that can transfer electrons with the gate⁵³ or the channel material⁹¹ (a detailed description of electrochemical reactions is given in the experimental techniques).

Ions are the main contribution to the current modulation by the gate electrode through the electrolyte, thus their variation can affect the device properties, making OECT an ideal candidate for ion sensing. For the far more common p-type depletion mode OECT, increasing concentrations of ions induce higher ionic charge at the interface with the channel, de-doping more effectively the semiconducting polymer. Ions sensing with OECT based devices has been proved for various cations⁹⁷ and in combination with ion-selective membrane^{98,99}.

OECTs can interface with biology as enzymatic sensors, and for the past 15 years they have been integrated with various enzymatic units for the detection of various analytes, spanning from healthcare to agricultural applications. The working principle of an OECT-based enzymatic sensor relies on current variation caused by local pH changes¹⁰⁰, or electron transfer to gate electrode^{83,93,101,102} or channel⁹¹. OECT enzymatic sensing has been explained in detail for a (p-type) glucose sensor by Bernardis *et al.*¹⁰³ Enzymes can catabolise substrates into electrochemically active products and enable redox reactions at the gate electrode (fig. 9a). When the product of the reaction is oxidised at the electrode there is a current decrease in

the channel, due to the semiconductor de-doping. In order to understand the physical process happening in the device we need to compare it with conventional electrochemistry, where the analyte detection is manifested in potential changes at the working electrode. In the OECT the electrolyte potential is determined by the capacitance at the electrode/electrolyte and channel/electrolyte interfaces (region 1). The addition of the analyte in the solution leads to electrons exchange with the gate electrode producing a potential drop at the interface (fig. 9b). Since the potential at the gate electrode is fixed, the change happens in the electrolyte potential (region 2), causing consequently a current variation in the channel.

Figure 9: a) Representation of an enzymatic based OECT, presenting an enzymatic unit at the gate electrode, that in the presence of the specific substrate undergoes redox reaction. b) Potential representations for the OECT gate/electrolyte and channel/electrolyte interfaces in the absence (1) and in the presence (2) of the enzymatic substrate.

The proper enzymatic biosensor design includes geometry and functionalization optimisation. Enzymatic sensing is affected by geometrical factors and Cicoira *et al.*¹⁰⁴ demonstrated that the sensitivity for an OECT peroxide sensor increased as the gate electrode dimensions decreased. The explanation comes from the potential drop at the two interfaces (fig 9b). For large gate, the potential drop at the gate/electrolyte interface is minimum, thus its variations are affecting less the electrolyte potential, when compared to small gate area. On the contrary, a small gate electrode induces larger current variations and should be preferable for developing enzymatic sensors. Modifications of the OECT electrode can also enhance the sensitivity. For example, deposition of nanoparticles^{92,105} or carbon nanotubes¹⁰⁶ can offer catalytic properties or easy functionalization surfaces to detect the analyte of interest. The enzymic units can be immobilised at the electrode surface, with chemical modification^{82,107} or entrapped in biocompatible membranes^{53,108,109}, which can also offer additional filtering and enhance the biosensor specificity^{84,110}.

OECTs have also been shown as effective platforms for immunosensors and nucleotide detection. Potential shifts happening at the gate electrode or in the channel can affect the semiconductor de-doping process and therefore be detected. When no redox reaction is involved with the addition of the analyte, the detection method relies purely on capacitive changes, and is considered non-faradic. OECT based immunosensors have been designed using various sensing units such as antibodies^{51,94–96}, oligonucleotide strands^{111,112}, or membranes¹¹³.

Biosensors, including OECTs, require biorecognition elements to selectively detect an analyte in solution. In biological processes, the interaction between proteins and molecules forms the basis of such selectivity. Enzymes have evolved in nature to selectively catabolise specific substrates and are thus used to design biosensors for the detection of specific metabolites. Likewise, antibodies (Abs) are proteins from the immune system able to recognise and bind external factors with high selectivity and specificity. For these characteristics they are the most used and studied proteins for molecular recognition in bioelectronic devices. Abs are the gold standard for diagnostic assays (such as enzyme-linked immunosorbent assay, ELISA); however, they present some disadvantages, such as batch-to-batch variability, low long-term stability, and difficult chemical modifications¹¹⁴. Engineered recognition elements are rapidly emerging as a solution to overcome Abs' limitations and are based on protein or oligonucleotide scaffolds. Aptamers, in particular, are oligonucleotide-based recognition elements which present high selectivity, facility of synthesis, and low-cost development¹¹⁵. These probes are designed from scratch through a technique called systematic evolution of ligand by exponential enrichment (SELEX)¹¹⁶. The SELEX method first selects an oligonucleotide sequence able to bind the target molecule, then amplifies it to enrich the nucleotide pool. Aptamers have already been successfully employed in many biosensing platforms, including electrochemical biosensors¹¹⁷, immunoassays¹¹⁸, and OECT-based biosensors^{119,120}.

OECT for *in vitro*/*in vivo* applications

OECTs have been coupled with living cells, from mammal to plant, as powerful tools to study electrical properties or for monitoring physiological processes. Since the current in the channel is strongly dependent on ion movement, OECTs have been used to monitor tissue integrity of living cells *in vitro*^{48,68}. OECT devices have also proven to be a versatile tool for drug screening^{121,122}, to record action potentials from cardiomyocytes with high spatiotemporal

resolution^{123,124}, to monitor cellular health¹²⁵ and after functionalization to record metabolite export in real-time⁹².

Implantable devices are gaining a lot of attention as powerful tools to record physiological processes in living organisms, revealing biological insight and allowing an overall investigation of the biological systems. Traditionally, *in vivo* recordings are performed with non-conformable apparatus, using rigid metal electrodes and probes or disruptive methods that create tissue damage and potentially alter local physiological processes^{126,127}. On the other hand, OECTs are proving their versatility for *in vivo* recording because of their unique characteristics. OECT based devices can be fabricated on ultra-thin or flexible substrates³², they can be miniaturised with high signal amplification, and they ensure high signal-to-noise ratio (SNR)⁷⁶. OECTs fabricated on flexible substrates have been used to record electrocardiography (ECG)^{87,128}, electrooculography (EOG)¹²⁹, and electroencephalography (EEG)^{32,88,130,131}. In parallel, ultra-thin OECTs with a more rigid encapsulation have been used to interface with the brain³³ or record neurotransmitters²¹. The integration of electronics, specifically OECTs, with the plant world is revealing a broad range of applications, from energy harvesting to plant physiology recording. In the first example, Stavriniidou *et al.* design an electronic circuit in a living rose, featuring OECTs as one of the main components¹³². In other cases, OECTs were interfaced with plants to record ion variations^{133–135} or metabolites¹⁰⁸.

CLINICAL BIOMARKERS

The OECT-based biosensors presented in this thesis were validated for the detection of biomarkers that are relevant for healthcare and agricultural applications, thus showing their high potential as a proper sensing platform. We developed sensors towards different analytes, ranging from small molecules to proteins.

Urea

Urea is the end product of protein catabolism; it is a small molecule (fig. 10a) that derives from amino acid deamination and ammonia conversion. Physiologically, it is dissolved in the blood and expelled by the kidney in the urine. Commonly, urea is measured in the blood as Blood Urea Nitrogen (BUN), or in the urine as a test for glomerular filtration. Urea, together with creatinine, is associated with various pathologies including chronic kidney disease (CKD), acute kidney injury (AKI), and kidney stones¹³⁶. In comparison with physiological concentration of urea in blood (5-10 mM), in CKD patients urea levels can increase up to 50-70 mM, making it a useful biomarker for diagnosis. Urea, as clinical analyte, is generally measured with a multistep enzymatic method that involves urea hydrolysis (fig. 10b) and ammonium quantification with dye indicator¹³⁶. Urea can also serve as an environmental biomarker to monitor water pollution¹³⁷, and its levels are also controlled for prevention of food (e.g. milk) adulteration¹³⁸.

Figure 10: a) Urea molecular structure, b) Urea hydrolysis reaction by the enzyme urease.

Uric Acid

Uric acid (UA) is the end product of purine catabolism (fig. 11a). The human body lacks the enzyme Urate Oxidase, necessary to metabolise UA, hence the metabolite is dissolved in the blood and expelled in the urine. Uric acid levels can be monitored to check for wound infections, because it can reveal the presence of bacteria in the exudate, therefore a possible bacterial contamination. UA is physiologically present in the wound exudate, under the form

of Urate in concentrations that vary between 220-750 μM ¹⁰¹. Some bacteria normally present on the skin can colonise the open tissue and give rise to infection. These bacteria, such as *P. aeruginosa*, can metabolise UA, decreasing its concentration in the wound exudate below 100 μM and making it an effective biomarker for wound infection. Abnormal UA levels in blood (higher than 400 μM) are also associated with bad nutrition (excessive purine intake) and metabolic disorders¹³⁹. These conditions are leading to gout and urate crystals precipitation in joints or other tissues, inducing inflammation. The gold standard techniques for UA detection are a colorimetric assay based on the reduction of the chromogen sodium tungstate and an enzymatic assay (fig. 11b) coupled to Uv-vis absorption spectroscopy¹⁴⁰.

Figure 11: a) Uric acid molecular structure. b) Uric acid catabolism by the enzyme urate oxidase

Interleukin 6

Interleukin 6 (IL6) is a multifunctional cytokine synthesised by a diverse class of cells, including mononuclear phagocytes, fibroblasts, and vascular endothelial cells. IL6 is a low molecular weight protein of 21kDa with an isoelectric point of 6.17, thus presenting an overall negative charge at physiological pH (fig. 12). IL6 has a pleiotropic effect, and it is involved in different pathways, from the immune reaction activation to the haematopoiesis¹⁴¹. IL6 is a pro-inflammatory agent, responsible for the acute phase response and an anti-inflammatory agent, inhibiting other cytokine releases¹⁴². Physiologically, IL6 is present in serum in femtomolar concentrations spanning from 100 to 200 fM¹⁴³. Increased levels of IL6 are associated with infection, tissue damage, and many chronic pathological states, such as cancer¹⁴⁴, chronic inflammation, and inflammaging¹⁴⁵. Recently, IL6 received a lot of attention because high levels (>3.5 pM) of this cytokine have been correlated with severe Covid-19 infection and respiratory failure¹⁴⁶. Cytokine detection and quantification are essential for precise monitoring of various diseases and because of their extremely low concentrations in bodily fluids, they require highly sensitive and selective detection methods. Typically, in

clinical tests, cytokines are quantified using enzyme-linked immunosorbent assays (ELISA) and antibody arrays, both methods requiring specialised equipment and trained personnel¹⁴⁷.

Figure 12: Molecular structure of interleukin 6 (PBD:1IL6)

Glucose

Glucose is one of the most widely quantified and studied clinical biomarkers. Glucose is part of the class of carbohydrates as a monosaccharide¹⁴⁸ (fig. 13a). It is the main source of energy for cells, and it plays an important role in metabolism regulation. It is stored in the form of glycogen in mammals as an energy reserve¹⁴⁸. Glucose is used as the main biomarker to diagnose and monitor diabetes, a metabolic disease that affects 9,8% of the world population¹⁴⁹. Diabetes is a long-term pathology derived from abnormal levels of insulin in the body¹⁵⁰. Insulin is a hormone produce by the pancreatic cells that daily regulates the glucose levels in the blood, allowing its absorption for energetic demands. Because of these daily insulin variations, glucose is a biomarker that needs to be detected and quantified regularly, for this reason, many methods have been developed to assist diabetic patients with portable glucose sensors¹⁵⁰. Commonly, glucose detection devices require a drop of blood (invasive methods) for the electrochemical detection of molecular oxygen, released as a result of the glucose catabolism by the enzyme Glucose oxidase (GOx)¹⁵⁰ (fig. 13b). Glucose detection can also help to anticipate epileptic seizures, due to sudden glucose concentration decrease for dysregulated metabolism in some parts of the brain¹⁵¹. Glucose is the main energy source not only of the animal kingdom but also in the plant world¹⁵². In contrast to mammals, which need to get glucose from external sources, plants can convert solar energy into chemical energy via photosynthetic process. During daytime, the plant stores the excess of sugars in starch granules in chloroplasts, while during night-time it is used as metabolic support^{152,153}. Glucose

also is an important signalling molecule in plants and is related to metabolism, stress responses, and growth¹⁵⁴, thus providing useful insight in plant physiology for agricultural applications.

Figure 13: a) Glucose molecular structure. b) Glucose catabolism by the enzyme glucose oxidase

Sucrose

Sugars, especially glucose and sucrose, are responsible for plant metabolism, cell signalling, and carbon allocation¹⁵⁵. In several tree species, sucrose is the main form of transported carbon because it is an efficient energy storage molecule, it is soluble in water, and it is not as highly reactive as glucose¹⁵⁶. In plants, sucrose (fig. 14a) is transported mostly in the phloem and in minor quantity within the xylem transpiration stream¹⁵⁷. Understanding metabolic pathways of sugars and their regulation can provide new biological insights into plant physiology. In order to address these questions, researchers need methods with high spatial and temporal resolution and real-time quantification. Sugar analysis in plants is commonly performed by invasive methods that imply the disposal of the organism or tissue after sample collection¹⁵⁸. Once collected, the sample is typically analysed using enzymatic assays (fig. 14b), mass spectrometry, or high-performance liquid chromatography to quantify sugars levels.

Figure 14: a) Sucrose molecular structure, composed by a molecule of glucose and fructose. b) Sucrose conversion till peroxide by three enzymatic units: Invertase, Mutarotase and Glucose Oxidase.

EXPERIMENTAL TECHNIQUES

Physical vapour deposition

Surfaces of non-conductive materials such as glass or plastic can be made electrically conductive depositing on the surface a thin layer of metal, using a technique called Physical Vapour Deposition (PVD). The PVD exploits the metal phase transition from solid to vapour and its deposition on a substrate, due to condensation. The PVD requires high vacuum to ensure a uniform film growth and to facilitate the vapour phase. The vapour phase can be achieved by treating thermally the metal in the chamber or through electron sputtering¹⁵⁹. In thermal evaporation, materials to be evaporated, are placed on specific holding boats or around resistive wire which are exposed to high direct current to increase the temperature and allow the material evaporation¹⁶⁰. The material deposition rate is controlled by adjusting the current passing through the holding boat and it is measured thanks to a quartz crystal microbalance. During the process, the material condenses both on the substrate and the crystal surface, estimating the thickness and the growing rate of the film. In this thesis, PDV has been used to evaporate thin layers of chromium and gold on PEN substrate in order to fabricate OECT.

Spin coating

Spin coating is a technique used to deposit a thin and uniform layer of soluble material on a solid substrate. A small amount of solution is drop casted in the centre of the wafer which undergoes fast rotation to spread uniformly the material all over the surface. Different thicknesses can be achieved using different spinning velocities and solution viscosities, the higher is the angular speed of the rotation the thinner would be the film. The solvents used are usually volatile and they partially evaporate during the spinning movement, leaving a solid material on the surface which can be further baked. In this thesis, spin coating technique has been used during the microfabrication process to deposit semiconducting polymer, photoresist and resist.

Photolithography

Photolithography is a process used to transfer a geometrical pattern to a substrate exploiting UV light. The process is commonly used in microfabrication because it allows to pattern

geometry with nm resolution. It requires specific photomask and photoresist which are sensitive to light. Photoresists can be positive or negative; in the first case the resist that is exposed to light became soluble to a developer, creating on the substrate the same geometry present on the mask. In the case of negative photoresist, the portion exposed to the light undergoes cross-linking becoming insoluble to the developer and producing on the substrate the negative image of the mask. Subsequently, the developed substrate is subject to an etching process, which can be chemical or physical, depending on the material to pattern. Chemical or wet etching uses solvents that can solubilise the uncovered material and removed it from the surface. Physical etching, typically used to remove polymer, is a process that uses reactive ions to bombard the uncovered surface of the wafer. In the end, the remaining resist is stripped away using the appropriate solvent and the substrate can be further processed with new layers. The photolithographic process as shown in figure 15 can be summarised by these steps:

Figure 15: Schematic of photolithographic gold patterning using a positive photoresist.

- 1) The wafer surface is carefully cleaned and dried to remove any particles which could lead to uneven photoresist deposition.
- 2) The resist material is spin-coated on the metal surface with a specific thickness, and it is baked to remove the remaining solvent.
- 3) The substrate is exposed to UV light through a photomask.
- 4) The photoresist exposed to UV light is removed using a developer reagent.
- 5) The underlying material is chemically or physically etched.

6) The remaining photoresist is stripped away chemically.

In this thesis, photolithography has been used for the fabrication of OECTs on PEN substrate. In more detail, the gold and chromium layers were etched chemically, while the PEDOT:PSS was physically etched using O_2 and CF_4 reactive plasma. The substrate was subsequently encapsulated using SU-8 2010.

Printing

Organic semiconductors are soluble in water or organic solvent, enabling their use in printing processes. Screen printing is a low-cost technique to pattern layers of organic or inorganic material in the form of inks on different substrates, including plastic and paper. This technique enables high volume and large-scale production, and the possibility to print on flexible substrate. The screen-printing process necessitates a frame that presents a screen mesh with the pattern to print, and a squeegee that presses the ink through the mesh till the substrate (fig. 16). Using-screen printing technique is possible to achieve a resolution of $75\ \mu m$ and a minimum thickness of $1\ \mu m$ ¹⁶¹. after the printing process the substrate is usually thermally cured, before proceeding with new layers. In this thesis, screen-printing technique has been used to fabricate OECT device with PEDOT:PSS as semiconducting material.

Figure 16: Schematic representation of screen-printing process. The ink is deposited on the screen and pushed through the mesh, transferring a specific pattern on the substrate.

Functionalization

Functionalization is a surface modification to enable interaction between the electronic interface and the biological surrounding. It includes processes to entrap or bind biological molecules that can respond to the analyte in solution. Biocompatible materials such as chitosan, gelatin or nafion are mostly used to entrap enzymatic units because they provide a stable and porous environment. While biological units such as antibody or oligonucleotide

strand can be attached through chemical reactions or using a self-assembly monolayer (SAM) with the proper functional groups. The functionalization strategies used in this thesis include enzyme entrapment in chitosan and porcine derived gelatines and thiol bonding between oligonucleotide strands and AuNPs.

Electrochemical deposition

Electrochemical deposition is a process used to cover a conducting surface with a layer of a different material, present in solution. An electrochemical deposition requires an electrochemical cell and involves electron transfer from/to electrodes to create layers or nanostructures. In the electrochemical cell two electrodes, an anode and a cathode are immersed in a solution containing the ionic compound of the material to deposit. When a potential is applied, positive ions migrate to the cathode and get reduced, accepting electrons, while negative ions travel to the anode getting oxidized. The potential applied determines the rate at which electrons are exchanged and can affect the morphology of the materials. Electrochemical deposition can be used to polymerise monomers, present in solution and create conductive material. For example, EDOT in poly(styrene sulfonate) solution can be oxidised to produce radical cations that will react with each other creating a film of PEDOT:PSS on the electrode¹⁶². In a similar way, metals can be deposited and shaped in nanostructure applying a potential to create a nucleation process that allows the formation of nanoparticles¹⁶³. In this thesis electrochemical deposition has been used to deposit a platinum layer on a printed OECT carbon gate electrode and to deposit AuNPs and PtNPs on a PEDOT:PSS gate electrode.

Cyclic Voltammetry

Cyclic voltammetry is a technique used to study the electrochemical properties of a system. It allows to characterise oxidation and reduction processes of species in solution and property of different electrode materials. The typical electrochemical set-up is composed of a Working Electrode (WE), a Reference Electrode (RE) and a Counter Electrode (CE) (fig. 17a). The WE is used to study electrochemical events happening in the cell. It requires well defined geometry and clean surface. The RE is used as a reference point to apply a potential at the WE and presents a stable equilibrium potential. The CE closes the circuit and allows a current flow between WE and CE. During cyclic voltammetry, a potential is applied from a potentiostat at

the working electrode, changing its energetic level and allowing electron transfer between electrode and electroactive species in solution. The plot in figure 17b is called voltammogram and represents the typical “duck shape” of a redox reaction. On the X-axis it is represented the applied potential, while on the Y-axis is represented the current passing at the WE. The electrode is scanned forward from negative to positive potentials, giving an anodic current, then reversely from positive to negative, recording a cathodic current. The peaks present in the voltammogram represent the oxidation peak in the anodic current region and the reduction peak in the cathodic one. These peaks are the result of electron transfer between species in solution and the WE ¹⁶⁴.

Figure 17: a) Potentiostat connected to an electrochemical cell. The magnification shows the set up to perform a cyclic voltammetry, composed by a working electrode (orange) a reference electrode (black) and a counter electrode (grey). b) Cyclic voltammetry curve for a reversible redox reaction.

An electroactive specie in solution presents an equilibrium state between reduce and oxidate state, described by the Nerst equation:

$$E = E^0 + \frac{RT}{nF} \ln \frac{(Ox)}{(Red)}$$

Where E is the potential of the electrochemical cell, E^0 is the standard potential of the species in solution. R and F are the universal gas and Faraday constant respectively, n is the number of electrons exchanged. T is the Temperature and (Ox) and (Red) are the oxidative and reductive specie concentrations. The typical “duck shape” of a cyclic voltammetry derives from the diffusion of the species from the bulk to the electrode. When the electrode is scanned forward the anodic current starts to increase, showing the oxidation of the specie

near the electrode surface. The current reaches the maximum point (oxidation peak) when the specie reaches the electrode also from the bulk via diffusion. The potential increase produces high quantity of oxidised specie next to the electrode, creating a layer that slows down the diffusion of reduced specie from the bulk, thus producing the current decrease. When the final potential is reached, the electrode is scanned reversely, and the cathodic current shows the reduction peak. From the voltammogram is possible to study the reversibility of the process, the number of oxidation states and electrode surface properties (such as capacitance). In this thesis, Cyclic Voltammetry has been used to study the AuNPs deposition on the PEDOT:PSS gate electrode.

Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) is a standardised technique that allows to study electrical component of a system, including surface and bulk properties. Compare to other electrochemical techniques, which study a steady-state of electrodes, the EIS allows separation of events on a time scale, because of the application of alternate current or voltage¹⁶⁵. Because of these features, EIS represents a valuable method to study surface modifications, such as biomolecule immobilisation and biding events. Electrical Impedance describes the resistance of electron flow, recorded applying alternate potentials in a small amplitude and recording a current with a phase shift (fig. 18). Impedance Z is the ratio between the voltage $V(t)$ and a phase shifted current $I(t)$ as a function of time.

$$Z = \frac{V(t)}{I(t)} = \frac{V_0 \sin(\omega t)}{I_0 \sin(\omega t + \varphi)}$$

$$\omega = 2\pi f$$

Where V_0 and I_0 are the voltage and current amplitude respectively, ω is the angular frequency given by the frequency f , t is the time and φ is the phase shift. As showed in figure 18, Impedance Z can be represented as function of the real impedance Z' and the imaginary impedance Z'' by the modulus $|Z|$ and the phase shift ϕ .

Figure 18: Diagram showing the phase shift between the voltage and the current in function of time. Impedance as complex value in a Z'' and Z' plot, given by the modulus $|Z|$ and the phase angle ϕ .

The term spectroscopy derives from the application of different frequencies to measure an impedance spectrum that allows to study many system parameters. Impedance can be displayed using two different plots: Bode plot, where $|Z|$ and ϕ are plotted in function of $\log f$ and the Nyquist plot which considers Z'' and Z' on y and x-axis respectively. In order to extract parameters that describe the system, the Impedance spectra is often compared to an equivalent circuit. The most common and simplistic circuit that describes the system is called Randles circuit (fig. 19) which consists in a solution resistance (R_s), in series with other elements, such as a Capacitance (C_{dl}) which describe the Electrical Double layer at the electrode interface, a charge transfer resistance (R_{ct}) and the Warburg element (W) to describe the diffusion.

Figure 19: Randles equivalent circuit of a simple electrode in contact with an electrolyte. The C_{dl} describes the charge at the electrode/electrolyte interface, R_s is the resistance of the solution, R_{ct} is the resistance to transfer of charge from the solution to the electrode (redox reaction) and the Warburg impedance to describe the diffusion from the bulk to the electrode surface. The Nyquist plot displays Z'' vs Z' and allows the extraction of the system parameters.

EIS can be used for analytical applications to detect molecules in the bulk. Biomolecules can be immobilised at the surface of the electrode to detect biological events, such as antibody-antigen binding. Commonly a small redox molecule, like ferrocyanide $[\text{Fe}(\text{CN})_6]^{4-}$, is added in the solution together with the analyte, to ensure a defined charge transfer resistance. When

the electrode surface is modified with molecules or sensing units the charge transfer resistance increases because the redox molecules are impeded from reacting with the electrode. When an analyte in solution binds a sensing element immobilised on the surface, it is possible to observe a further increase in R_{ct} , therefore binding events can be detected following the behaviour of the charge transfer resistance. Alternately they can be detected following C_{dl} variations. Molecules present at the surface can induce changes in the thickness and in the charge of the electrical double layer.¹⁶⁶ In this thesis, EIS has been used to study the functionalization and the analyte binding of the AuNPs/PEDOT:PSS gate electrode.

Scanning electron microscopy

The optical microscope is a powerful tool to study surface modifications. However, light diffraction limits the resolution of this technique. Higher resolution can be obtained using Scanning Electron Microscopy (SEM). SEM takes advantage of accelerated electrons to achieve a shorter wavelength, compared to visible photons, and a better resolution. SEM represents an advanced tool to study surfaces and sample features. The sample is scanned by an electron beam in a high vacuum environment. The sample surface sends signals back to a detector which can describe the morphology, the topography and the composition of the analyzed area with a spatial resolution of 1 nm. The sample needs to be electrically conductive and grounded to avoid surface charge accumulation, which may lead to artefacts and poor resolution. The SEM working principle relies on the interaction between the electrons from the beam and the sample. This interaction can be elastic and non-elastic. The non-elastic interaction occurs when an incoming electron (from the beam) collides with the electric field of the sample atom, resulting in an electron expulsion from the valence band and X-ray radiation emission. This electron, called Secondary Electron (SE), presents low energy and comes from the most superficial part of the sample, generating the topographical image. Elastic interactions instead represent collisions where the incoming electron deflects back from a deeper region of the sample. This electron is called Back Scattered Electron (BSE) and presents a different direction, but a similar energy. SE, BSE and X-ray are detected from specific detectors to form an image and provide useful insight about sample composition and morphology. In this thesis SEM has been used to study the morphology of the PEDOT:PSS gate electrode modified with AuNPs.

Fourier transform Infrared spectroscopy

Matter interacts with the whole electromagnetic spectrum, from the gamma ray to X-ray and specific wavelengths can be used to study its composition. The Fourier Transform Infrared Spectroscopy (FTIR) uses the infrared region IR ($4000 - 600 \text{ cm}^{-1}$) to gain useful information. An infrared spectroscopy irradiates the sample with an IR beam recording the molecular absorption made by functional groups. Molecular bonds which present a dipole are responding to infrared, because of their natural vibrational frequencies. When these bonds interact with IR, they increase vibration and oscillation intensity absorbing specific wavelength radiations. The radiations are collected by a detector to create an interferogram and subsequently translated to a spectrum using the Fast Fourier Transform function. An IR spectrum plots the light intensity versus the wavenumber (cm^{-1}), measuring the amount of light absorbed by each different functional group at specific wavelength. The absorbance spectrum is calculated as:

$$A = \log \left(\frac{I_0}{I} \right)$$

Where A is the absorbance, I_0 is spectrum background intensity, and I is the sample intensity spectrum. The sample preparation for this technique is essential. Since plastic and glass can absorb in high quantity the IR radiations, the cell for the measurement is made of ionic material such as potassium bromide (KBr). To overcome some limitations of the traditional FTIR, the Attenuated Total Reflection ATR can be used. In this set up, sample doesn't require any preparation and measurements are achieved fast without interruption. The ATR uses a crystal, most commonly made of zinc selenite (ZnSe), on which the IR ray hits with a 45° angle, and it is reflected to the interface between the crystal surface and the sample. This generates evanescent waves which undergo numerous internal reflections and get attenuated where the sample absorb energy. The IR beam then exit the crystal and is collected by the detector. In this thesis the FTIR has been used to ensure the presence of oligonucleotide strands on the AuNPs/PEDOT:PSS gate electrode.

Electrical measurements

Source Measure Units (SMUs) are instrument used to record the OECT electrical characteristics and measure the biosensor detection range. SMUs are able to supply a potential to the system in analysis, while measuring a current. To characterise a transistor there are two main measurements to perform: the transfer and the output curves. The transfer curve (fig. 20a) is measured by keeping fixed the voltage between source and drain and sweeping the voltage at the gate electrode. The output characteristics is obtained by sweeping the voltage between source and drain for fixed gate voltages (fig. 20b).

Figure 20: a) PEDOT:PSS transfer characteristic obtain keeping fixed the voltage between source and drain at -0.3V and sweeping the gate voltage from -0.2V to 0.8V. (b) OECT output characteristic obtain sweeping the source drain voltage from 0V to -0.8V for different gate voltages.

To prove the sensitivity and the range of detection of an OECT biosensor transfer characteristics can be used. The transfer characteristics are firstly performed in a control buffer until stabilisation, then the gate electrode is incubated with increasing analyte concentrations and recorded until current stabilisation. Another method measures keeping fixed both the voltage between source and drain and the gate voltage. The current is continuously recorded and increasing or decreasing concentration of the analyte are consecutively added to the sensing area. In this thesis electrical measurements have been used to assess the analyte sensing of the OECT, in vivo and in vitro.

Green house

Plants are grown inside a greenhouse to monitor and keep constant the growing conditions. The green house provides a well define photoperiodic condition and a controlled humidity. In

this thesis the green house has been used to growth the Tobacco plants and the Poplar trees for the in vitro and in vivo experiment.

SUMMARY OF PAPERS

Paper I

Label free urea biosensor based on organic electrochemical transistors.

In this paper we developed a biosensor for the detection of urea. Urea is an important biomarker not only in medical diagnosis, where it is an indicator of kidney and liver disfunction, but also in food safety and environmental control. We demonstrated a urea biosensor using the enzymatic unit urease entrapped in a gelatin hydrogel, onto a fully printed PEDOT:PSS-based organic electrochemical transistor (OECT). We showed a response against increasing urea concentration in phosphate saline buffer (PBS), with a limit of detection as low 1 μ M and a time of response of 2-3 minutes. The sensing mechanism relies on the modulation of the channel conductivity induced by the ionic species that are produced from the urea hydrolysis. We fabricated the OECTs using screen-printing technique on flexible substrates to ensure a significant reduction in manufacturing time and costs making these enzymatic OECT-based biosensors as appealing candidates for monitoring of urea levels at the point-of-care or in the field.

Contribution: device printing, device characterization

Paper II

Flexible Printed Organic Electrochemical Transistors for the Detection of Uric Acid in Artificial Wound Exudate.

In this paper we developed a low-cost sensor, that can provide real-time monitoring of wound infection. The sensing platform can be used as a tool to improve chronic wounds management, at the hospital and at home. In particular we used a printed organic electrochemical transistors (OECT) biosensor for monitoring the analyte uric acid (UA), which is correlated to bacterial infection in wounds, and we demonstrated its detection properties using artificial wound exudate. We developed the biosensor exploiting the enzymatic catalysis of UA to 5-hydroxyisourate, by Uricase. We entrapped the enzyme in a dual-ionic-layer hydrogel membrane onto the gate to ensure selectivity. This dual membrane can filter and hinder possible interference analytes that will lead to nonspecific faradic reactions, for example, the direct oxidation of UA. The biosensor showed a reproducible response in phosphate buffer

saline and in complex solutions mimicking the wound exudate with a high sensitivity for the pathological levels of UA in wounds (<200 μM) and exhibited a limit of detection of 4.5 μM in artificial wound exudate.

Contribution: experimental and device design, master student experiment supervision, manuscript writing.

Paper III

Organic electrochemical transistor-based aptasensor for interleukin-6 label-free detection.

In this paper we developed an OECT based biosensor for the detection of interleukin 6 (IL6), an important biomarker associated to various pathological processes, including chronic inflammation, inflammaging, cancer and severe COVID-19 infection. We ensured the selectivity of the biosensor against IL6 using aptamers as sensing units, engineered oligonucleotide strands, able to bind specifically the cytokine of interest. We developed an easy functionalization method based on gold nanoparticles deposition onto the PEDOT:PSS gate electrode for the subsequential electrodeposition of the thiolate aptamers. We proved a detection range from picomolar to nanomolar concentrations for IL6 and the selectivity of the device when compared to the detection of Tumor Necrosis Factor (TNF), an analogous cytokine whose levels are also high in inflammatory processes.

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

Paper IV

Diurnal in vivo xylem sap glucose and sucrose monitoring using implantable organic electrochemical transistor sensors.

In this paper we described the first-ever demonstration of a molecule detection through an enzyme reaction chain and the first-ever direct sensing inside biological tissue via implantable organic electrochemical transistor. We showed for the first time an OECT-based sucrose and glucose sensor directly implanted into the xylem tissue of poplar trees (*Populus tremula* x *tremuloides*), a tissue responsible for transpiration. We report a real-time, *in-vivo* monitoring of sucrose and glucose variations over 48 hours. We demonstrate that the OECT sensor can

operate in in-vivo environment, and that it can detect important biological metabolites directly from the tissue of interest, monitoring relevant biological event over time. As a demonstration of the proof-of-concept and the kind of biological insights this new technology enables, we observed previously uncharacterized diurnal changes in sucrose levels in the xylem sap. Our technology outperforms conventional methods, making it an attractive tool for studying biological processes in general, without interfering with the system physiology.

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

Paper V

Real-Time Monitoring of Glucose Export from Isolated Chloroplasts Using an Organic Electrochemical Transistor.

In this paper we reported a biosensor based on organic electrochemical transistors (OECT) as an useful devices for real-time monitoring of plant biological processes. OECTs can interface directly with the electrolyte enabling the detection of biological events and at the same time, providing a fast and amplified signal. So far, these devices have mainly been applied to cells or body fluids for the development of clinical tools to use in diagnostic. We showed a first and direct detection of biomolecules from organelles using an OECT glucose sensor applied to chloroplasts, which are the plant organelles responsible for the light-to-chemical energy conversion of the photosynthesis. We proved real-time monitoring of the exportation of glucose for two distinct metabolic phases (day-time and night-time) and we quantified the dynamic exportation with a time resolution of 1 min, demonstrating a monitoring dynamics an order of magnitude better than conventional methods.

Contribution: major contribution in the experimental part including microfabrication, measurements, data analysis and manuscript writing.

CONCLUSION AND OUTLOOK

This thesis provides a description of organic based biosensors, developed using organic electrochemical transistors as the sensing platform, for the detection of five different analytes relevant for both medical and environmental applications. We focused on developing functionalization strategies to transform OECTs in selective sensors for specific analytes and we prove their detection in complex media and in *in vivo* system. We used two fabrication methods to develop the OECT based biosensors: a cost-effective method, using screen-printing techniques in order to have disposable and portable devices for home care or in-field applications, and an ad hoc design, using microfabrication techniques for more selective targets. We studied various factors and functionalities of the OECT to optimize the functionalization method and the sensing range, achieving highly effective biosensors for all the applications.

Looking forward, we continue working on developing innovative enzymatic detection methods exploiting the properties of specific enzymes to enable direct electron transfer (DET) together with novel conductive materials that can be polymerized through biological reactions. DET can open major advantages in biosensor development, such as the absence of toxic mediators or toxic byproducts, and the operation at voltages close to zero, which avoids undesirable parasitic redox reactions and enables an even lower power consumption¹⁶⁷. DET based biosensors have the potential to become a candidate for *in vivo* applications. However they present many challenges regarding functionalization and enzyme optimization¹⁶⁸. I believe OECTs and organic based devices, because of their fabrication techniques, materials, and operating conditions are promising platforms for personalized systems for molecular diagnostic. However, the OECT sensors require standardization and enhanced reliability before they can become point-of-care system or wearable devices. Moreover, they will require integration with e.g., microfluidic systems, integrated circuit, and portable power supplies for their use as wearable/implantable devices.

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