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CICLO XXXII

Printed organic electronics: towards sensing and biosensing

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Abstract

This work is aimed at impacting on the sensing and monitoring of bio-signals and biomolecules relevant to assess the state of health of people possibly improving the state of the art where the available instruments generally require complex analytical methods commonly implemented by dedicated equipment in a hospital setting. Large research efforts are in progress in research labs to develop and qualify friendly usable devices that could better implement homecare and personalized monitoring aiming at intervening as early as possible to maintain the best health possible for each person.

Within this ambitious and quite challenging framework, this PhD project is contributing by developing biosensors that show quite relevant features in terms of flexibility, stretchability, conformability and biocompatibility. Features that are quite essential for devices that should be easily and efficiently interfaced with the human body. Our devices belong to the well-known family of Electrochemical Organic Transistors (OECT) and were implemented by an innovative manufacturing protocol, i.e. the Aerosol Jet Printing (AJP) technique, on flexible/stretchable biocompatible substrates.

We chose AJP technique since we believe and hence demonstrate that could be applied to a wide range of printable inks allowing the fabrication of in-line all-printed devices with high definition features.

We hence have first of all studied the printing parameters for all the needed components including contacts, active channel on different substrates, optimizing them to achieve the best definition and functionality of the materials. For the active channel we studied poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), that could be considered the benchmark polymer in Organic Bioelectronics. We fabricated OECTs using two different flexible substrates: polyimide (Kapton) and a fibroin film. We demonstrate that we can achieve state of the art performance of our printed devices compared to similar ones in terms of architecture and materials used. For the most advanced bio-electronic sensing device, we selected fibroin film as a substrate because not only shows better biocompatibility than a Kapton foil but we could also achieve a functionality of great relevance: optimized fibroin films give a particularly good skin self-adhesion, improving also signal transmission without the need of the commonly used gels, features that Kapton doesn't offer without the addition of gels and other

Our OECTs printed on fibroin have hence been directly applied on the human skin for ECG signal monitoring in a standard clinical configuration.

This represents the most important achievement: the development of a fully-printed OECT on a flexible fibroin substrate that could monitor of ECG signals both *in vivo* and real time giving signals amplitude even better with respect to commercial electrodes with comparable time resolutions.

These results are a very good basis for the future planned activities aimed at developing devices with an increased quality response for directly monitoring bio signals generated by neuronal activity.

We have also explored skin-attachable optical sensors. To this aim, we have studied fibroin photoelastic properties that are fundamental considering the continuous mechanical stimulation induced by the human body during movement. We made experiments on the two major fibroin phases (Silk I and Silk II) by applying longitudinal strains on a silk fibroin whispering gallery mode (WGM) cavity fabricated on an optical fiber taper.

In synthesis the major relevant results shown in this thesis, concern hence materials preparation, inks development, printing optimized processes, all printed device fabrication, that have been used to develop and qualify OECTs on fibroin as very promising all printed devices for biomedical applications. We also explored fibroin itself as a base material for optical sensors, achieving the feasibility towards developing wearable optical sensor for biomedicine and rehabilitation.

We show the feasibility of fully biocompatible, cheap, easy to handle and scalable systems that could contribute to the development of wearable and all-day user electrophysiology and optical sensors. We are working to further improve the devices by increasing their sensitivity and time response mainly working on the gain and noise suppression. The idea is to possibly extend the fields of applications to neural signals, intrinsically faster and less intense than ECG, to electrophysiology, and to wearable optical sensors.

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Chapter 1

The present chapter is dedicated to the description of the most recent achievements of electronic devices as a sensor and biosensors with a particular focus on the bio signal monitoring. Section 1.1 describes the born of the bioelectronics with a particular focus on the description of the three major application fields. Section 1.2 describes the rise of the Organic Bioelectronics that presents new properties to overcome inorganic bioelectronics limits. A brief overview about the story and the working principles of organic and semiconductor polymers has been reported in Section 1.2.1.

Section 1.2.2 describes the born and the electrical properties of PEDOT:PSS, the benchmark polymer in Organic Bioelectronic; Section 1.3 reported the working principle and the properties about Organic Electrochemical Transistors (OECT) that are selected as sensors in our work. Section 1.3.1 discusses the theoretical model developed for understanding OECT working principles. Section 1.3.2 discusses the use of OECT as sensors and biosensors with a particular focus on OECT as chemical and biochemical sensors (Section 1.3.2.1), bio sensors (Section 1.3.2.2) and for bio signal monitoring (Section 1.3.2.3).

1.1 Bioelectronics

Bioelectronics can be defined as the study of the synergic interaction between biological systems (cells, neurons, organs, DNAs....) and electrical devices.

Luigi Galvani pioneering experiments on bioelectricity, performed in 1780 at the University of Bologna, are widely acknowledged as the starting point of the modern Bioelectronics.¹ Galvani himself, introduced for the first time, the concept of “animal electricity” and hence probed the use of bioelectric forces to study living systems.

The explosion of Bioelectronics as an effective and better established research field has been taking place during the last century, when several electronic devices have been rationalized and developed determining major impacts in biology and medicine. Presently, electronic devices are commonly used in modern hospitals and represent fast developing precious tools for perspectives of healthcare. For example, defibrillators are used to save an uncountable number of lives every year, electrocardiograms (ECG) and electroencephalograms (EEG) recording represent an indispensable system for early cardiac and neuronal diseases.³

Recent developments have been explored taking advantage of organic materials aiming at extending the range of Bioelectronics towards applications where conventional electronic devices based on inorganic standard electronic are not recommended.

Bioelectronics and organic bioelectronics applications can be classified in three main areas (Figure 1.1):

- **Drug delivery:** To achieve the desired therapeutic effect limiting side effects, pharmaceutical drugs must be delivered in safety with the appropriate concentration. Safety and efficacy are the most important requirements for a drug delivery system¹, and nowadays several applications are reported in pain therapy after surgery² and general disease treatment^{3,4}.
- **Biosensing for chemical- and bio- markers:** The possibility to functionalize electronic devices with biomarkers opens the route to biosensing applications. Biosensors are devices that combine the functionality of a bioreceptor with a transducer system to determine the amount of a biomolecule (biomarker) in a biological system and fluids.⁵ According to IUPAC a biosensor was definitely as “*An independent integrated receptor transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element.*”⁶ Biosensors with several transducers and different types of bioreceptors⁵ are used, for instance, in glucose detection (this is the first biosensor application)⁷, enzymatic sensing and immunosensors.
- **Bio-signal recording:** The electrical activity of cells, tissues and organs is based on fluxes of ionic species. Ionic currents monitoring such activity is the best and most common way to determine their integrity and their functioning and Bioelectronic devices represent an ideally viable solution to analyze and measure such signals but also an effective way to stimulate specific responses and functionalities. Typical devices described in literature are Electrodes^{7,8} and Thin Film Transistors (TFTs)^{9,10} as well as ionic pumps.¹¹ Depending on the specific application and the type of bio-environment, Bio-signals monitoring often requires good conformability and good biological interfaces/compatibility by the dedicated devices. The most important physical parameter to determine a good quality of signals recorded by electrodes is the impedance at the electrode interface. Electrical impedance is a measure of materials attitude at responding to the application of alternating electrical currents of various frequencies. Electrodes with a low impedance are desirable to achieve high quality bio-signals recording and a low impedance can be realized both by increasing electrode area and using low impedance materials such as, for example, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS)¹² as electrodes coatings.

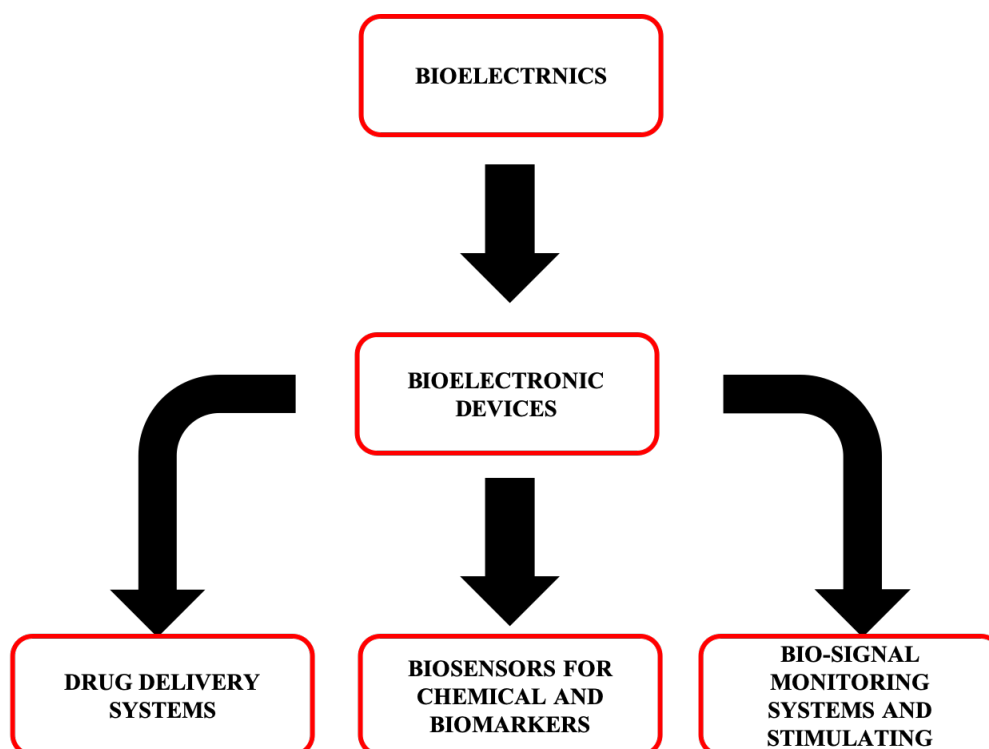


Figure 1.1 Bioelectronics major devices typology

Local and site specific drug delivery as an alternative to the more classical systemic administrations. It is a fast developing field that is based on the novel strategies boosted by the engineering of nanocarriers and nano-systems loaded with proper drugs and vehicles towards the specific site. On the other hand, biosensing, biosignaling recording and biostimulation are typically implemented by electronic devices. The miniaturization of semiconductor devices as well as the development of new classes of biocompatible materials (organic semiconductors, biopolymers^{5,6}....) and fabrication techniques (roll to roll ⁷, inkjet ^{8,9,10}, aerosol jet printing ¹¹) are opening new opportunities in biomedical and commercial applications. Organic semiconductors and polymeric substrates, combined with innovative deposition and fabrication techniques, make also possible the development of fully flexible and conformable devices. ¹² Moreover, the integration of biosensors and drug delivery systems favor the device implantation and take advantage from wearability in terms of in situ diseases treatment and bio-signals recording and stimulation. ^{13,14}

To give an indication of the evolution of the field, in Figure 1.2 the number of publications per years of bioelectronics and organic bioelectronics applications is shown. (source: Web of Science™ Database)

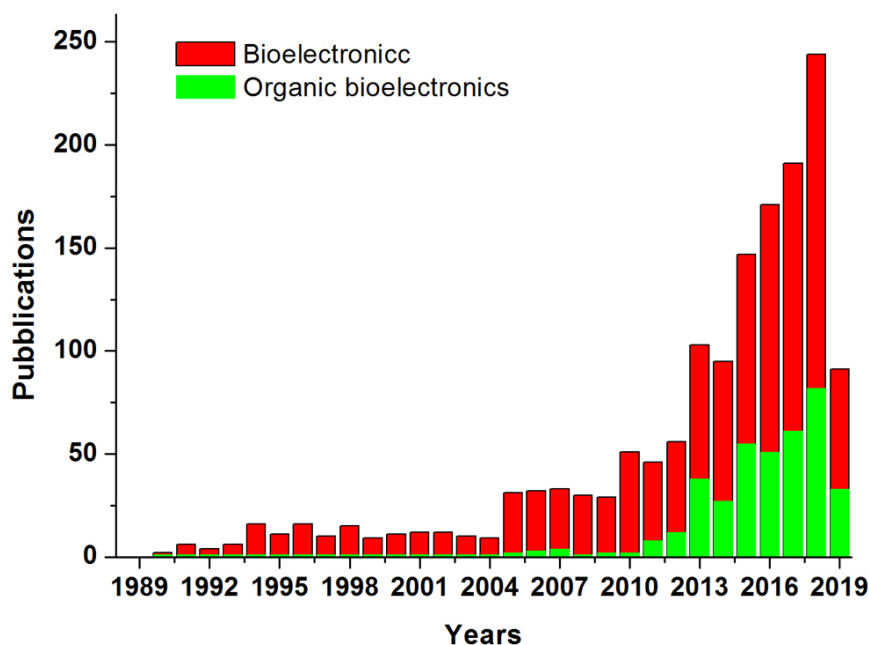


Figure 1.2: Evolution of the number of publications per year in the field of Bioelectronics. Relevant increase is observed around the 2005. More recent is the development for organic bioelectronics. An increase in number publication in 2013 is reported.

1.2 Materials: from inorganic to organic

Materials choice plays a critical role in bioelectronics. Bio-compatibility is obviously the first requirement in order to interface electronic devices with biological systems whenever the direct contact is needed. Ideally bio-signaling rely on the ion (ionic are in-fact most of the bio-processes) to electron (our fantastic electronic world) transduction that is best made possible by materials that make simple the transduction of ionic to electronic signals.

Nonetheless, traditional inorganic devices have been the first being used in this field, even though ionic conduction is intrinsically, poor even if present at all, in inorganic crystalline conductors. The first most relevant studies in the field are based on the use of conventional electronic devices consists of the fundamental pioneering work carried out by Fromhertz. In particular, in 1991 he reported the first silicon field effect transistor (FET) coupled with neuron.¹³ In this study, a neuron is being successfully supported in close contact with the insulating layer of a the gate electrode immersed in an electrolyte. By applying the needed polarization voltage between the source and drain the current driven in the transistor channel resulted to be modulated by the neuron-Si interaction so that, under

microelectrodes stimulation, its activity could be directly monitored. This fundamental work has then given rise to a blossoming literature on neuron dynamics studies.

One of the main goals of the current research is to achieve efficient and sensitive *in vivo* recordings of bioactivity in order to study bio-functionalities and hence diagnose diseases at their early stage. To this end, on one hand, to minimize stresses and interferences with the bio-system under study, conformable and flexible devices are desirable. The biological world is a “soft world” and the Young Modulus of silicon is six orders of magnitude higher with respect to bio-materials (around 100 GPa, while the skin, for example, shows a modulus around 13 MPa ¹⁴). On the other hand, ionic conductors represent the natural interface to communicate with living beings.

To overcome these critical issues, several research groups worked to develop innovative and alternative materials. Organic insulators and semiconductors have proven to fulfill to the above requirements. Several devices based on organic materials for sensing applications are indeed reported in literature.^{15–17} Organic materials present wonderful and useful characteristics suitable as an active material in bioelectronic devices:

- Flexibility/stretchability ¹⁸
- Mixed electronic-ionic conduction
- Easy functionalization
- Solution processability (spin casting, 3D printing)
- Mimic of biological systems with self-assembling properties
- Possibility to decorate with biomolecules to promote cells viability
- In thin-film state are transparent and allows transmission imaging

1.2.1 Conductive and semi-conductive Polymers

Conductive and semi-conductive polymers, or intrinsically conductive or semi-conductive polymers (ICPs), are organic materials able to conduct an electrical current. ¹⁹ In literature several examples of conductive polymers are reported. The most popular ICPs are reported in Fig. 1.3

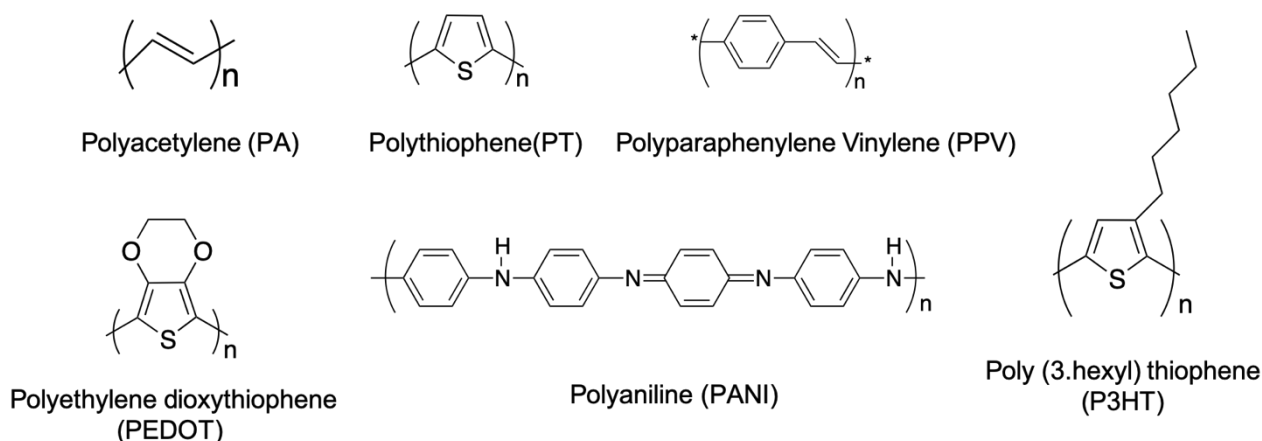


Figure 1.3: Example of major conductive and semi-conductive polymers reported in literature

IPCs show a conjugated molecular structure with a sequence of single (σ -bond) and double bonds (π -bond) along the polymeric backbone. ICPs are basically composed of carbon atoms with a sp^2 hybridization, composed by three σ bonds and a p_z orbital. The overlap between p_z adjacent orbitals create a p delocalized state along the polymeric chain. This delocalization promotes a main route for charge mobility along the polymer backbone. A double band structure, π -band and π^* -band²⁰, is achieved by increasing the number of double bonds.

The energy difference between the highest occupied molecular orbital (HOMO), i.e. the highest occupied state in π -band, and the lowest unoccupied molecular level (LUMO), i.e. the lowest unoccupied state in the π -band, is named energy gap (E_g). Typically for organic polymers the E_g is higher (in the visible range) than that of inorganic semiconducting materials and comparable to that of dielectric materials. For this reason, for long time polymers have been considered insulating materials.

This view changed in 1977 when Shirakawa²¹ (Nobel Prize for Chemistry in 2000 together with Heeger and MacDiarmid) produced a highly conductive polyacetylene (PA) upon doping this material by exposing it to bromide and iodine vapors. The electrical conductivity was 10 order higher than that of the undoped PA monomers.

Starting from this result, a big effort has been dedicated to increase the conductivity of π -conjugated polymers. Chemical or electrochemical procedures have been found to be effective in promoting a reversible doping of conducting polymers. This kind of doping is quite different from that of inorganic materials, where doping is generally promoted by interstitial impurities, i.e. neutral atoms infiltrated into the semiconductor lattice.²²

Chemical doping is implemented by a redox reaction causing the oxidization (p-type doping) or reduction (n-type doping) of the polymer by electron acceptors or donors.^{23,24} The electrochemical doping occurs by oxidation or reduction reactions assisted by an electrode causing the ions to diffuse in or out of the polymer in order to compensate for the electrical charge on the polymeric backbone.²⁵ Chemical doping shows some limitations that have been resolved recurring to the electrochemical doping. Through the latter procedure, the doping yields are finely controlled by the voltage applied between conductive polymer and electrodes; in addition, the doping effect via the electrochemical mechanisms is generally reversible and allows achieving both p-type and n-type doping without introducing doping species via chemical reactions.

The charge injected by doping processes generate some defects localized along the polymeric chain that induce new energy levels in the electronic structure including polarons and bipolarons that are involved in the conduction mechanisms.¹⁹

ICPs can be further classified as polymers with degenerate or un-degenerate ground state. Polyacetylene (CH_x) can present two cis and trans isomeric forms.²⁶ The trans-polyacetylene is a typical example of polymer with degenerate ground state, and it is characterized by two isoenergetic forms A and B as reported in Figure 1.4.

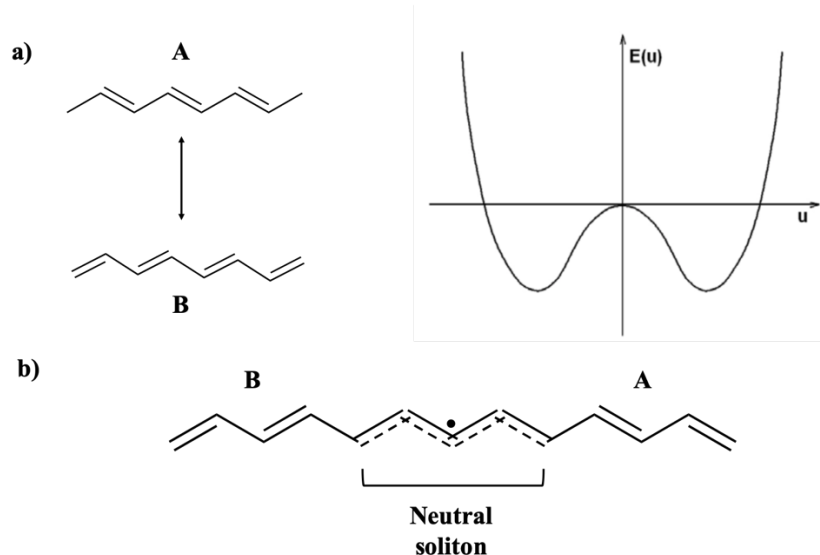


Figure 1.4: Schematic description of soliton in PA. In a) the phase A and B of the trans-polyacetylene is reported. In the energetic diagrams the two energy curves of the degenerate phase A and B are shown. b) reports the structure of soliton that is between the phase A and B

These two levels create a defect in the electronic-lattice system that is formed at the interconnection point between the A and B forms. This defect is called soliton and creates an extra level in the middle

of the energy gap.²⁷ This level can be full (positive soliton), empty (negative soliton) or with only one electron (neutral soliton) in the mid gap level.

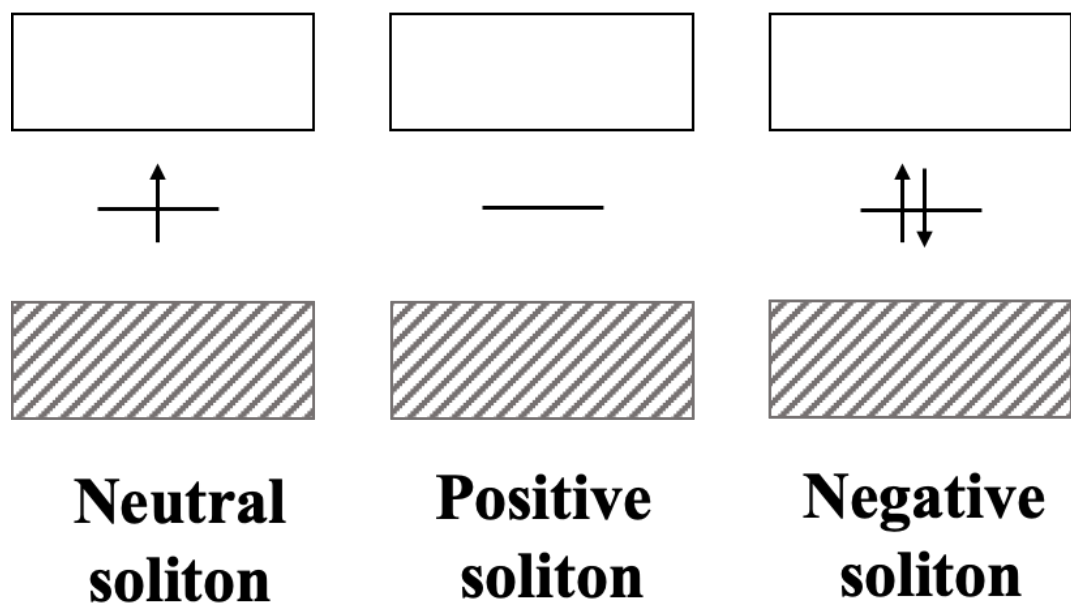


Figure 1.5: Schematic of energy level in a soliton structure. The degenerate energy level is positioned in the middle of the band gap. Neutral (only one electron), positive (no electrons) and negative solitons (energy level full of electrons) are reported

Most of conjugated polymers, for example, polythiophene (PT), do not show a degenerate ground state but two resonance structures, i.e. the aromatic and the quinoid structure. These structures do not have the same energy, being the quinoid the most energetic structure. Hence, a non-degenerate ground state is achieved. This means that a couple of energy levels are generated in this case.

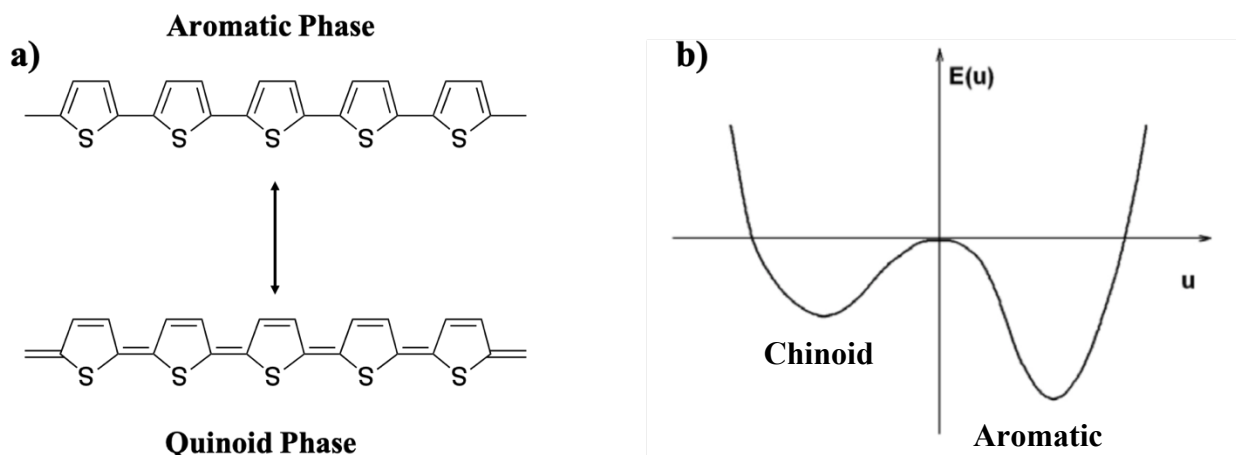


Figure 1.6: Schematic of conductive Polythiophene. In a) the aromatic and chinoid phase is reported. These phases are not degenerate and in the corresponding energetic graph is shown in b).

Injection or removal of electrons by doping process generates a defect along the polymeric chain that is named polaron. Polaron is a radical- ion couple, negative or positive, confined between the quinoid and aromatic forms. In case of positive polarons, an electron is removed from the HOMO level while in case of negative polarons an electron is added in the LUMO level.

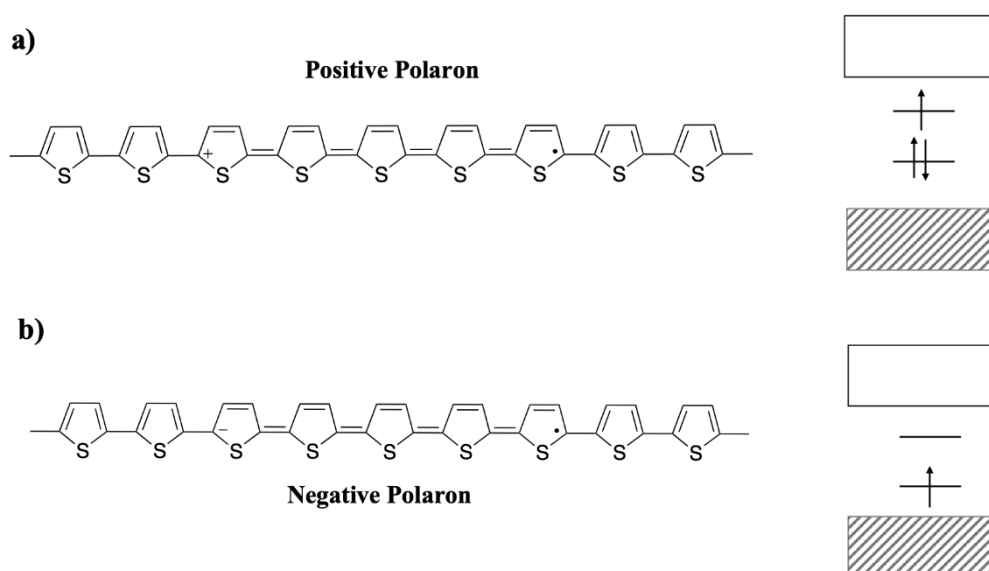


Figure 1.7: Schematic diagram of polarons structure in a Polythiophene. In a) the positive polaron structure is reported. In the energy diagram on the right in the figure, the energetic levels present one full level and one with only one electron. In b) the negative polaron structure is reported. For this one energy level is empty and one with only one electron.

An extra addition of charges may induce the formation of two coupled polarons, i.e. a bipolaron (Fig.1.8).

The number of polarons and bipolarons increases upon doping levels increasing. At high doping levels, new energy levels are formed in the band gap, reducing it as a consequence of the overlapping among single polaronic energy levels, promoting thus a conductivity enhancement due to electric field assisted charge carriers drift along the polymeric chain.

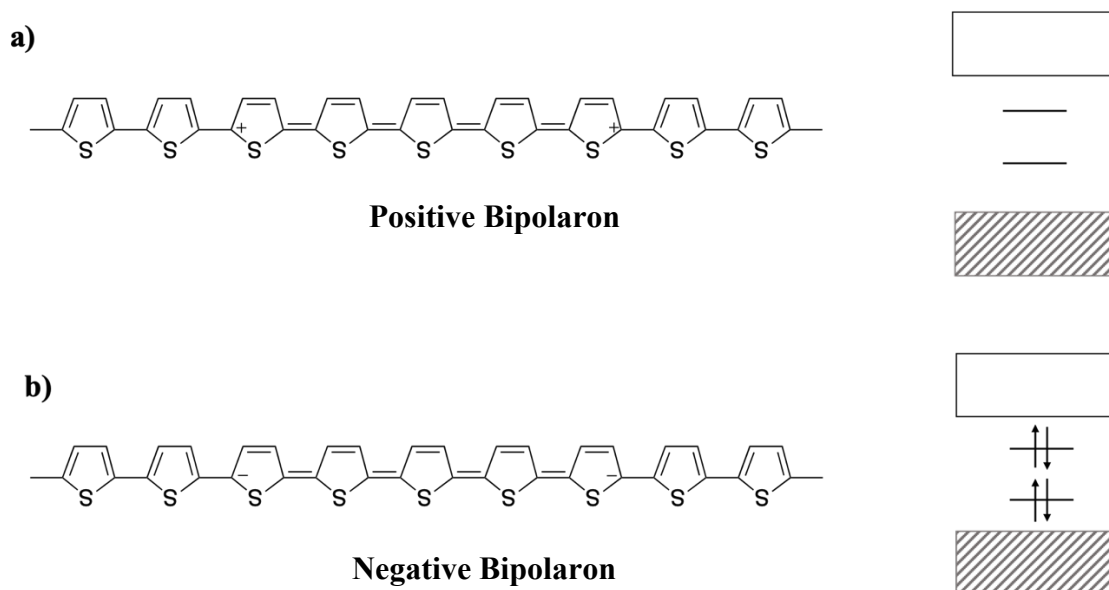


Figure 1.8: Schematic of a bipolaron structure. In a) the positive bipolaron is shown together with the corresponding energy levels structure on the right side. In b) the negative bipolaron with the corresponding energy levels structure is reported

1.2.2 PEDOT:PSS

Doped polyacetylene was considered for a long time as the prototype most highly conductive polymer. The main challenge in the development of a technically useful and industrially viable product is intrinsic to some polyacetylenes' properties such as its air sensitivity to the point that it loses the doping level upon exposure to air.

The first promising approach to overcome polyacetylenes' drawbacks was to stabilize the sensitive π -electron by heteroatoms in electron-donor substituents. For the first time, it has been realized in polyaniline by incorporating both nitrogen N and sulfur S atoms in the heterocyclic conjugated

structure, which became the most promising substituents.²⁸ The electron donating function of N and S ring atoms stabilizes, in fact, the π -conjugated system.

A new era for ICPs started when in 1982 Taurillon and Garnier²⁹ observed an electrical conductivity in Polythiophene. Thiophene was electropolymerized with perchlorate or tetrafluoroborate counterions, and a 10-100 S/cm conductivity was achieved.²⁹ The real breakthrough in IPCs world was the discovery that oxygen substituents at 3- and 4- position in the thiophene moieties are able to stabilize the doped form, thanks to their electron donating properties. This discovery disclosed the era of highly conductive, stable and processable thiophene-based polymers, among which Poly(3-hexylthiophene-2,5-diyl) (P3HT) and Poly(3,4-ethylenedioxythiophene) (PEDOT) are the most representative ones.

In particular, PEDOT is the most known π -conductive polymer, thanks to its air stability and high conductivity. PEDOT was synthesized for the first time in 1980 using a standard oxidative chemical polymerization EDOT moieties.³⁰

An insoluble powder with a high conductivity was accordingly obtained. Its insolubility represents a problem for the processability. A solution is adding counterions during the reaction to balance polymer charge and to achieve solubility. Several counterions have been tested (e.g. PSS^- , ClO_4^- , BF_4^- , NO_3^- , SO_4^- , etc.), and their choice is limited only by their solubility and stability in reaction conditions.

Polystyrenesulfonic acid (PSS) is the counterions used to form a polyelectrolyte complex (PEC) with PEDOT, where PSS is the polyanion and PEDOT the polycation.³¹ Water is used as solvent for PEC synthesis and a stable and industrial scale dispersion is achieved. Nowadays, aqueous PEDOT:PSS dispersion is commercialized under the commercial name of BaytronTM by Bayer AG and CleviosTM by Heareus. PEDOT:PSS is the most known stretchable conductive polymers used in electronic field. Its combined properties including biocompatibility (FDA approved), high stability, film-forming formation, flexibility and ionic and electronic transport, PEDOT:PSS make it the most commonly used conductive polymer for several devices such as organic strain sensors^{32,33}, organic field effect transistors (OFETs) and especially in organic electrochemical transistors (OECTs)³⁴⁻³⁶.

In PEDOT:PSS suspensions PSS is always in excess with respect to PEDOT, and the weight ratio of thiophene groups to sulfonic acid groups in standard PEDOT:PSS dispersions falls in the range between 1:2.5 to 1:20.

Both conventional and innovative techniques can be used to prepare PEDOT:PSS films. A PEDOT:PSS dispersion can be deposited in principle by all common techniques for generally used for solution processable polymers, such as spin coating, spray coating, but also electrodeposition methods. Nevertheless, other innovative printing techniques such as screen printing³⁷, inkjet printing³⁸ and aerosol jet printing³⁹ have been explored and studied to deposit PEDOT:PSS in its film form.

The dispersion is typically described as being composed by gel-like particles that are composed by a polyanion PSS-rich shell and a PEDOT-enriched core. In the case of uniform films, the typical structure formed has been depicted as a “pancake-like morphology”, where there is a succession of PEDOT and PSS regions.⁴⁰ For amorphous polymers and in particular for PEDOT:PSS, the conduction mechanism is commonly described in terms of charge hopping between adjacent sites (PEDOT cores) separated by thin insulating layers (PSS shells). This means that the conductivity is morphology dependent.

Pristine PEDOT:PSS films are characterized by an electrical conductivity (σ) not exceeding 1.0 Scm^{-1} ;^{41,42} an increase in electrical conductivity can be achieved via the secondary doping procedure, consisting of the addition of organic polar solvents to the PEDOT:PSS suspension^{43,44,45}, or by post processing treatments by the use of strong acids^{46,47}, ionic liquids^{48,49} etc.

Fan et al⁵⁰ proposed a graphical model to understand the morphologies difference between the pristine PEDOT:PSS films and the treated ones. In this model, as reported in Fig. 1.9b, an amorphous configuration is used to describe the pristine PEDOT:PSS film's composition. The amorphous configuration is constituted by PEDOT-rich grains. This configuration can be changed by the above treatments into a highly conductive one, characterized by much more ordered and wider PEDOT cores separate by thinner PSS shells (reduction of PSS amount). The ordered PEDOT chains are bonded with PSS molecules by ionic interactions

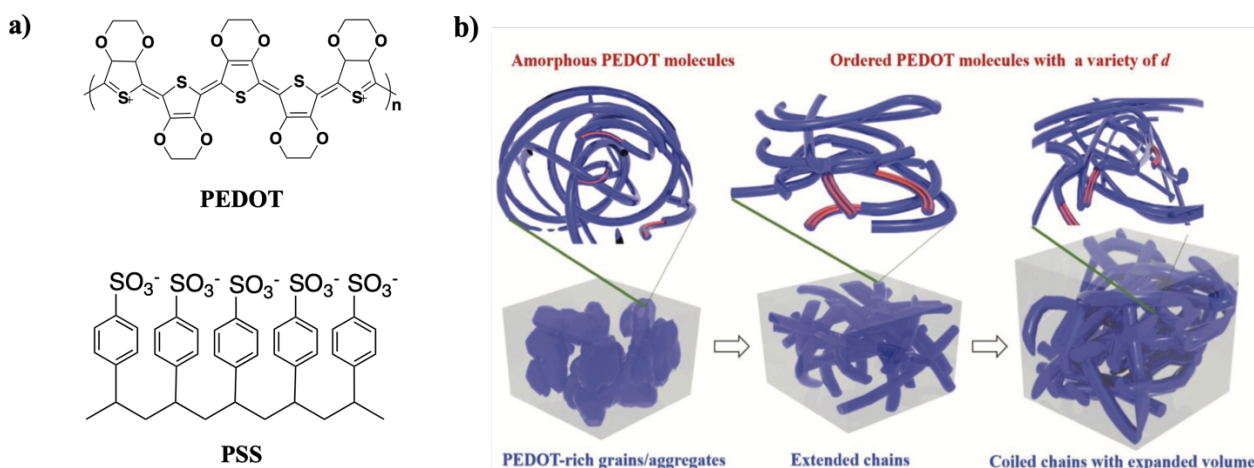


Figure 1.9: In a) schematic PEDOT:PSS chemical structure is reported. In b) the PEDOT:PSS structure transition due to doping and post-treatment procedures with PSS removed.⁵⁰

Several secondary dopants have been reported in literature: ethylene glycol (EG) ^{51,52}, glycerol, dimethyl sulfoxide (DMSO) ^{53,54}, D-sorbitol ^{55,56} and ionic liquids ⁵⁷.

In 2002 Zhang et al ⁵⁸ reported a PEDOT:PSS anode adding D-sorbitol and glycerol into the polymer dispersion. An increase in conductivity by two orders of magnitude (1 to 100 Scm⁻¹) was achieved in this case. Kim et al ⁵⁹ reported the use of DMSO as secondary dopant. A 3 wt% of DMSO addition increased the cohesion among molecules in the cast film (PSS barrier thickness decreasing). ⁶⁰

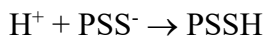
Post processing treatments are also used to increase conductivity. Ouyand et al ⁶¹ demonstrated an increment in electrical conductivity by immersing PEDOT:PSS film in an EG bath. The electrical conductivity was increased from 0.4 to 200 Scm⁻¹ due to a combined effect consisting of a transition from amorphous configuration to an ordered structure and an enlargement of PEDOT cores provoking an enhancement of interchain transport into PEDOT-rich domains.

Aleshin et al ⁶² showed a conductivity dependence from the temperature described by the Mott's equation, typical of a Variable Hopping Range (VHG) conduction mechanism:

$$\sigma(T) = \sigma(0) * \exp \left(- \left(\frac{T_0}{T} \right)^\alpha \right) \quad \text{Eq. 1.1}$$

where $\sigma(0)$ is the infinite temperature and $T_0 = 16 / (k_b N(E_f) \xi^3)$ for $\alpha = 1/4$. Here ξ is the distance from a site where the electron wave function to decay to 1/e of its value, often referred to as the localization length and $N(E_f)$ is the density of states. For this reason, an annealing process around 120°C for few minutes is needed after film deposition. The thermal post deposition treatment induces a rearrangement of the film with a morphology consisting of a change of PEDOT chains form quinoid to benzoic structure. A strong phase separation between insulating PSS and the PEDOT conductive regions is achieved and the PSS excess is removed.

A strong acid post treatment is the process that enhances electrical conductivity of PEDOT:PSS films. In this procedure H⁺ ions are formed due to strong acid effects provoking the formation of PSSH, a neutral form upon the combination between H⁺ ions and PSS⁻ molecules.



Thanks to PSSH formation, a new rearrangement in these structures is achieved. The PSSH molecule confined inside the film structure was then removed by rinsing the film in pure water. ^{63,64}

1.3 Organic electrochemical transistor (OECT)

After his discovery in the mid-1984,⁶⁵ Organic electrochemical transistors (OECTs) became one of the most interesting and largely reported transistor architectures in literature, because of their versatility in terms of applications in several fields. In particular, OECTs are promising and effective candidates for creating an interface with the biological world. The low operating voltage (below 1 V), its amplification of the ion-to-electron transduction combined with relevant electro-mechanical and optical properties of PEDOT:PSS, the material most used to fabricate OECTs active channels, make OECTs a well suitable device architecture for biological applications, including bio-sensing and bio-signal recording.

A typical OECT is made of an organic semiconductor film (the channel) in contact with an electrolyte (in liquid or solid state) in which an electrode, the gate electrode, is vertically immersed in or put in contact with it (the planar configuration).

The organic semiconductor film is in contact with two (metallic) electrodes, the source and drain electrodes, that define the geometry and dimensions of the polymeric channel such as reported in Fig 1.10.

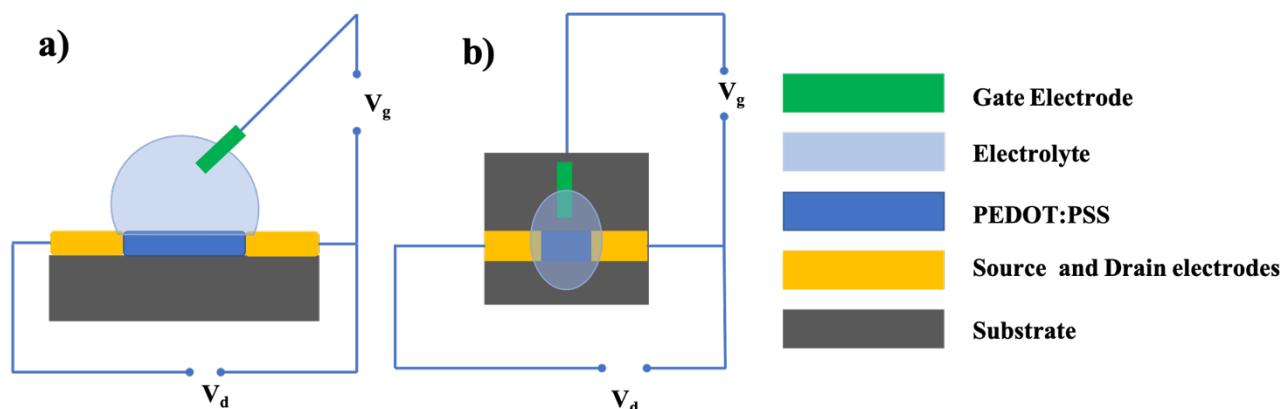


Figure 1.10: Schematic of an organic electrochemical transistor (OECT) structure. In a) is reported the set up with the vertical gate electrode, while in b) the planar gate electrode set up is shown.

OECTs operation is controlled by two voltages applied to the drain (drain voltage V_{ds}) and gate (gate voltage V_{gs}) terminals, being the source terminal grounded. The drain voltage induces a current (drain current I_{ds}) which is proportional to the density of free holes and electrons drifting in the channel. The V_{gs} controls the injection of ions from the electrolyte to the permeable organic channel, thus controlling the channel doping-dedoping properties (conductivity enhancement-reduction).

PEDOT:PSS represents the benchmark organic p-type semiconductor (positive charge carriers, holes) used for fabricating the OECT channel.

OECTs can operate in two modes, depletion and accumulation respectively.⁶⁶ In the depletion modes, typical for PEDOT:PSS based OECTs, an hole current is registered in the channel upon application of V_{ds} and in the absence of V_{gs} ; this state has been defined as the ON-state (in these conditions the channel is degenerately doped). When a positive voltage is applied to the gate electrode, cations are injected from the electrolyte into the polymeric channel. In this case a decrease in current is achieved as a function of the gate voltage due to the compensation of an excess of negative charges induced by PSS on PEDOT sites (dedoping). Hence, the current reduces reaching its minimum value (Fig.1.11a); now the device is in its OFF-state.

In the accumulation mode, the device is in its OFF-state. When a negative voltage is applied to the gate, an anion injection is achieved. In this way the number of holes increases and the transition from the OFF state to the ON state is achieved (Fig.1.11b). Due to the strong swelling and porosity of the polymer, the doping process is a typical bulk process in OECTs involving the whole material, unlike the case of organic field effect transistors (OFETs) where the conductivity change is just an interfacial phenomenon. All the active volume of the channel can be hence involved during the doping-dedoping process, and in this way, it is possible to reach large current modulations with very low applied V_{gs} .

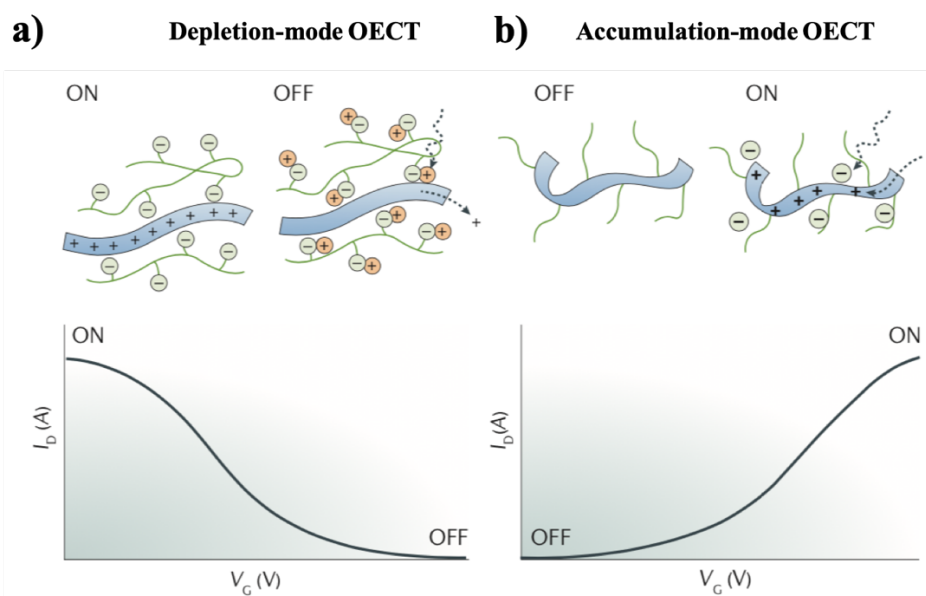


Figure 1.11: Schematic of depletion and accumulation mode in a typical OECT. The transfer curves for the two operation modes are also reported. In the depletion mode a positive voltage applied to the gate electrode induces a decrease in the drain current, instead in the accumulation mode if a negative voltage is applied to the gate an increase in gate current is registered.⁶⁶

The classical manufacturing process used to fabricate OECT is based on the well-known optical lithographic technique ^{67,68} in which the pattern is transferred to the film from a mask using a photoreactive material (photoresist) that can react under exposition to the UV-light. This technique presents some limitations such as the number of steps needed, the poor compatibility with several organic materials and the limited number of substrates that can be used. The introduction of printing techniques for devices manufacturing has fully changed the fabrication paradigm.

In 1994, the first electrochemical transistor partially fabricated using printing technologies has been reported.⁶⁹ Screen printing technique has been used to deposit carbon-based source and drain electrodes and dielectric on flexible substrate (PVC).

In the early of 1990 PEDOT inks are started to use for devices manufacturing. ⁷⁰ Only in 2002 the first application of PEDOT:PSS print in an OECT devices is reported. ⁷¹ The sensors were fully realized by printing and coating techniques on flexible substrates such as paper and plastic foils. This is a turning point, after which the use non-conventional substrates such as fibers ⁷² or non planar substrates has created unprecedented opportunities for manufacturing OECT on large scale and with inline processes on flexible substrates.

1.3.1 OECTs performances

OECTs transduce small ionic signals via the application of a gate voltage into enhanced I_{ds} currents. This process is typically described by the transfer curve, where the I_{ds} is plotted as a function of the applied V_{gs} . The device transconductance (g_m), calculated by performing the first derivative of I_{ds} with respect to the gate voltage and represents the figure of merit describing the transduction efficiency. Transconductance in OECTs is mostly due to the volumetric nature of the device response.⁷³

The high achievable transconductance makes OECT devices ideally suited for monitoring very small biological and physiological signals.

OECT operation was firstly modeled by Bernard et al⁷⁴ by introducing a well-known theoretical model.

According to the functioning scheme mentioned above and generally accepted, it is assumed that ions from the electrolyte are injected into the polymeric channel generating a change in the channel current. Accordingly, the device can be considered as the composition of two circuits: one ionic and the other electronic.

The electronic circuit describes the hole current in an organic semiconductor film from the drain to the source electrode and is modeled by the Ohm's equation:

$$J(x) = q\mu p(x) \frac{dV(x)}{dx} \quad \text{Eq. 1.2}$$

Where J is the current flux, q is the elementary charge, p is the hole density, μ is the holes mobility and $dV(x)/dx$ is the electrical field.

The Bernard model describes both the steady state and the transient OECT performance. In the OECTs' steady state the hole mobility is considered constant, while the material composing the channel is considered impurity and defect free. The geometry of the conductive channel is shown in Fig. 1.12a. The ionic circuit is described by a capacitor in series with a capacitor, describing the flow of ions in the electrolyte and the storage of ions in the channel respectively (fig 1.12b)

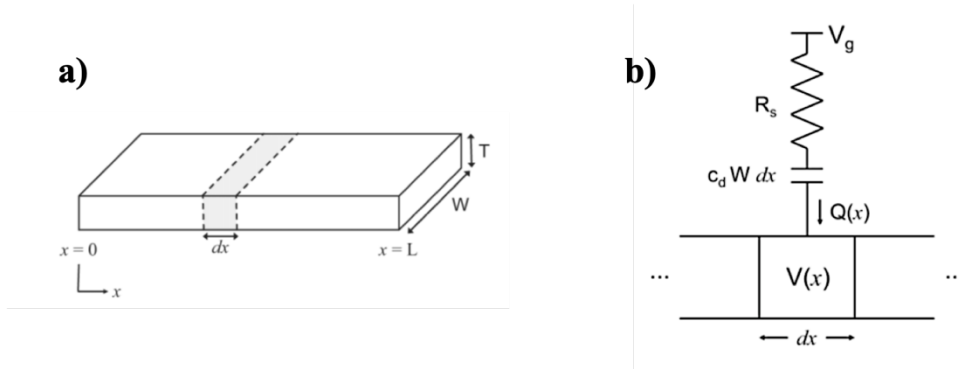


Figure 1.12: Geometrical sketch of the structure used in the model. In a) the semiconductor film is reported, where $x=0$ is the contact point and $x=L$ is the film length. In b) the structure of ionic and electric circuits is combined in the semiconductor film.⁷⁴

The equation describing the OECT steady state is given in Eq.1.3

$$J(x) = q\mu p_0 \left[1 - \frac{V_{gs} - V(x)}{V_p} \right] \frac{dV(x)}{dx} \quad \text{Eq. 1.3}$$

When $V_{ds} < 0$ is applied between drain and source electrode, the Eq.1.3 can be spitted in two part and two different regimes are achieved:

$$I_{ds} = G \left[1 - \frac{V_{gs} - 1/2V_{ds}}{V_p} \right] V_{ds} \quad \text{for } V_{ds} \geq V_{ds}^{sat} \quad \text{Eq. 1.4}$$

$$I_{ds} = -G \frac{V_{ds}^{sat^2}}{2V_p} \quad \text{for } V_{ds} \leq V_{ds}^{sat} \quad \text{Eq. 1.5}$$

Where V_{ds}^{sat} is the voltage applied to reach a current in saturation regimes and G is the conductance of the organic film. V_{ds}^{sat} is the difference between V_{gs} and V_p where V_p is the pinch off voltage.

G and V_p are dependent on the geometry of the channel.

$$G = \frac{q\mu p_0 W t}{L} \quad \text{Eq. 1.6}$$

$$V_p = \frac{q p_0 t}{c_d} \quad \text{Eq. 1.7}$$

Where t , W and L are the thickness, width, length of the channel respectively. And p_0 is the initial hole density, and c_d is the capacitance for area unit.

Bernard model also describes the OECT transient behavior. The transient is dominated by two phenomena: the injection of cations in the channel and the removal of holes induced by the neutralization caused by the injected cations.

In a simplified condition, when a constant gate voltage is applied, the transient behavior is consistent with the steady state current. Under these assumptions, the transient behavior for a simplified OECT is reported as:

$$I_{ds}(t, V_g) = I_{ss}(V_g) + \Delta I_{ss} \left(1 - f \frac{\tau_e}{\tau_i} \right) \exp \left(-\frac{t}{\tau_i} \right) \quad \text{Eq. 1.8}$$

where $I_{ss}(V_g)$ is the steady-state source-drain current at a gate voltage V_g and $\Delta I_{ss} = I_{ss}(V_g=0) - I_{ss}(V_g)$. In this equation, two different time constants are introduced, τ_i and τ_e . The time constant for the ionic transport in the electrolyte τ_i is dominated by the solution resistance and the capacitance of the ionic double layer. In the Guy-Chapman double layer theory the capacitance is dependent on the channel length and on the ionic concentration according to the equation $\tau_i \sim L/C^{1/2}$. To increase the time response an increase of electrolyte concentration or a decrease in channel length is necessary.

The time constant for electronic transport (τ_e) is instead relevant for the transient response. The electronic current depends on the ratio τ_e/τ_i , defined as

$$\frac{\tau_e}{\tau_i} = \frac{lL^2}{\mu V_{ds}} \quad \text{Eq. 1.9}$$

where l is the position of the gate electrode, L is the channel length and μ is the hole mobility.

The Bernard model gives a good fit to interpret the output of an OECT device and a quantitative prediction of the transconductance g_m in the form:

$$g_m = \left(\frac{W}{L}\right) t \mu C^* (V_{th} - V_g) \quad \text{Eq. 1.10}$$

Where, again, W , L and t are the channel width, length and thickness respectively, while μ and C^* are the charge mobility and the capacitance for unit volume of the channel, respectively. From Eq. 1.10 a dependence of g_m from the channel geometry is evinced.^{35,75}

The channel thickness is an important parameter to adjust the optimal value of g_m for sensing and bio signal recording applications. Recently, D'Angelo et al.⁷⁶ not only confirmed that the transconductance value increases with the thickness t at V_{ds} , but also demonstrated that g_m is increase with the channel voltage.

In 2010 Tarabella et al.⁷⁷ demonstrated a dependence of the current modulation on the materials used as gate electrodes. They used a typical PEDOT:PSS based OECT device in combination with a NaCl 0,1 M solution used as electrolyte to study the effect of Ag and Pt gate electrodes. They demonstrated an increase in the OECT response with the Ag gate electrodes with respect to Pt ones. This experiment confirmed a transition from an operating capacitive or non-Faradaic regime in the case of Pt gate electrodes, to a regime known as Faradaic in the case of Ag gate electrodes. In the non-Faradaic regime, V_{gs} induces a transient current in the electrolyte and a double electrical layer (EDL) is formed at the electrolyte/electrode interface. In the Faradaic regime, a redox reaction at the gate electrode promotes a higher steady-state current in the electrolyte.⁷⁸ Hence, while EDL formation corresponds to an effective reduction of the applied gate voltage along the electrolyte, the onset of the Faradaic current better assists the injection of cations into the PEDOT:PSS channel, thus inducing a faster and higher current modulation in OECTs. In addition, in the Faradaic regime there is not any measurable dependence of current modulation upon changing the gate area, while in non-Faradaic regime the OECT response depends strongly on the gate surface area, as reported in Fig. 1.13.

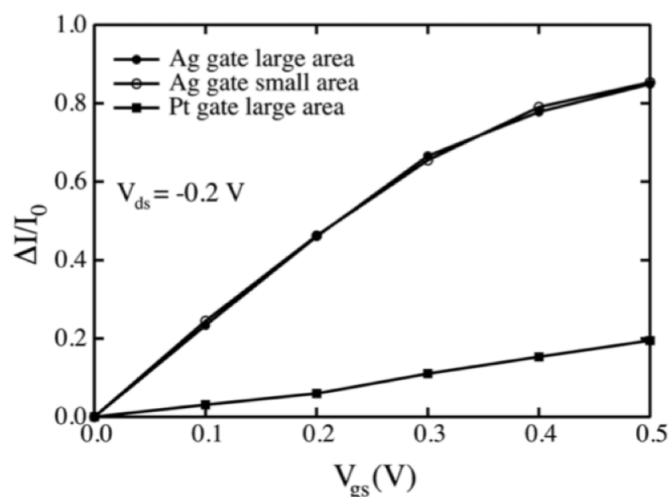


Figure 1.13: The transfer curve for Ag gate with two different areas (3.3 mm^2 and 1.6 mm^2) are practically the same with an overlap of the experimental points. This phenomenon supports the hypothesis that redox reactions at the gate electrode generate a steady state source-gate current which dominates the performance of the OECT.⁷⁷

Finally, it is worth to note that the response time strongly depends on the electrolyte nature. As expected, in the case of solid electrolytes the device time response is slower with respect to that of OECTs based on liquid electrolytes.⁷⁹

1.3.2 OECT Applications in bioelectronics

Since its discovery in 1984, as described in the previous sections, OECT devices have been intensively studied due to their interesting properties such as low operating voltage, good amplification, good ion to electron amplifying transduction and good interface with biological systems. The research works dealing with OECTs' applications is presently very large and steadily increasing in particular in the fields of chemical biochemical sensing, including, more recently, quite impressive works where OECTs' have been demonstrated to be ideally suitable for bio-signal monitoring.

1.3.2.1 OECT as Chemical sensors and Bio-chemical sensors

In 1985 Wrigthon et al ⁸⁰ demonstrated that an OECT based on polyaniline (PANI) could efficiently sense redox reagents. PANI is a polymer that modified its conductivity as a function of PH values, in

fact this polymer can switch from the un-conductive to the conductive form by a change in the pH of the electrolyte solution. For this reason, the proposed device also showed a pH sensitive response in terms of current changes.

Later on, OECT with a polyaniline has been used by Dabke et al.⁸¹ for detecting metal ions such as K^+ , Ba^+ , Na^+ and Ca^{2+} . The devices are composed by a polyaniline doped by ethers, able to detect K^+ ions with at a lowest concentration of 10^{-7} M thanks to the generation of defects in the polymeric backbone. A new perspective was open by Bergrenn et al.⁸² that developed an OECT with a PEDOT:PSS active layer for selective Ca^{2+} sensing. A Ca^{2+} -selective ionophore deposited on a polymeric membrane on the top of the PEDOT:PSS channel and was used to selectively determine Ca^{2+} concentration in a liquid medium. A sensitivity for concentration around 10^{-4} M was achieved in this case. Starting from the same concept, Mousavi et al.⁹³ used selective membranes in OECT devices for selective sensing of K^+ , Ca^{2+} and Ag^+ with lowest concentration limits around 10^{-4} M, 10^{-4} M and 10^{-5} M respectively.

The great relevance of these results in the implementation of selectivity in a device that is not intrinsically selective for different ions even though other strategies were tempted. The first systematic study on implementing ion selectivity in a OECT has been reported by Lin et al.⁸³ They analyzed PEDOT:PSS based OECTs in sensing K^+ , Ca^{2+} and Al^{3+} with different ionic concentrations in the electrolyte. First, a KCl 0.1 M solution and Ag/AgCl have been used as electrolyte and gate electrode, respectively. The transfer curves for the different cationic concentrations have shown a slow shift of the effective gate voltage (the gate voltage at which the PEDOT:PSS dedoping is actually effective) to lower values upon ions concentration increases.

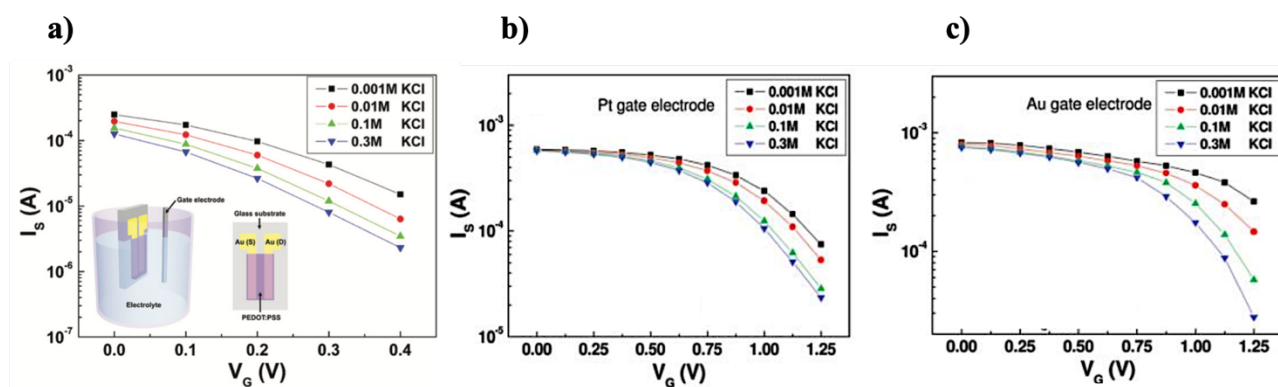


Figure 1.14 In a) transfer curve for several KCl concentrations measured with an Ag/AgCl gate electrode. In the inset the schematic of the devices used. In b) and c) transfer curves for Pt and Au gate electrodes are reported. No Nernstian trend is observed by the authors for Pt and Au electrodes.⁸³

In addition, they demonstrated the dependence of ion selectivity from the type of the gate electrode used during the experiment. The observation was that the transfer curve recorded using an Ag/AgCl (Fig.1.14a) electrode shifted to lower gate voltage values upon increasing the cationic concentration following a Nernstian trend. Meanwhile, for electrodes based on noble metals (Pt and Au) (Fig.1.14b and Fig.1.14c) higher ion sensitivities have been obtained due to the interface EDL capacitance at the gate/electrolyte interface.

1.3.2.2 OECT as Bio-sensors

Detection of glucose and determination of its level in solution is the most studied and reported application of OECTs operated in sensing mode. Contractor et al.⁸⁴ demonstrated the first glucose sensor based on a polyaniline channel over which they immobilized glucose oxidase. The observed dependence of the film conductivity to the glucose concentration in the electrolytic solution, hence the sensing mechanism, was attributed by the authors to the pH change in the region near the active layer due to the glucose reaction with the glucose oxidase and a 2 mM of detection limit has been achieved.

Zhu et al.⁸⁵ demonstrated a simple glucose sensor based on a OECT device at neutral pH. The device was made by PEDOT:PSS and a Pt wire as active channel and gate electrode, respectively and a Phosphate buffer saline solution (PBS) has been used as electrolyte. When glucose is added to the PBS solution, the channel current decreased dramatically due to the interaction between glucose and glucose oxidase enzyme (GOx) immobilized on the active channel.

Macaya et al.⁸⁶ reported an OECT glucose sensor with a limit of detection of a few micromoles, which is within the range of human level. PEDOT:PSS and a Pt wire were used as active channel and gate electrode respectively. In contrast with the previous works, the GOx was not immobilized on the polymer but dissolved together with glucose in PBS electrolyte. In this way, a direct reaction between glucose and GOx takes place into the electrolyte, resulting in improving the device limit of detection thanks to the Faradaic current generated by the H₂O₂ formation.

In the 2009 Shim et al.⁸⁷ reported a new class of glucose sensors: an all-plastic OECT on PEDOT:PSS that has been used both as active layer and as gate electrode, in this second case previously functionalized by ferrocene mediator. Ferrocene in fact can transfer electrons from the GOx to the gate electrode giving rise to. A detection below the micromolar level has been achieved with this kind of geometry.

Starting from these results, several research groups devoted efforts on the development of full PEDOT:PSS OECTs. Among these, Gualandi et al.⁸⁸ reported an all-plastic PEDOT:PSS OECT for

selective dopamine sensors. The detection limit was only about 6 μM which is still higher respect to the real amount in human fluids. The milestone of this work is the presentation of a flexible and selective sensor for dopamine.

OECTs devices have been used for sensing several kinds of analytes such as DNA strands ^{89,90}, dopamine ⁸⁸, interleukin and other biomolecules associated to the arising of degenerative diseases. ⁷⁶ The proven biocompatibility of PEDOT:PSS allowed the use of OECTs as cell-based biosensors. Yan et al ⁹¹ reported the first cell-based OECT biosensor. They demonstrated the possibility to grow cells on the PEDOT:PSS active layer. The sensing mechanism was attributed to the electrostatic interaction between PEDOT:PSS and the confluent cell layer. Specifically, the presence of cells on the active layer shifted the transfer curve towards more positive gate voltages, due to the negative charge on the cell surface.

The cell-based OECT is useful for *in vitro* monitoring of the cells growth mechanisms and to investigate the cells activity. For example, Romeo et al ⁹² developed a cell-based OECT biosensor for *in vitro* monitoring of the cellular activities, specifically to monitor death mechanisms of tumor cells under treatment with specific drugs. The OECT has been implemented in this case by a Transwell (Twell) in which the A549 human lung cancer cells are cultivated on a porous membrane and exposed to the anticancer drugs doxorubicin. Even better sensitivity for cell death monitoring, validated by a model developed ad hoc, is achieved when compared to typical biological sage (Fig 1.15).

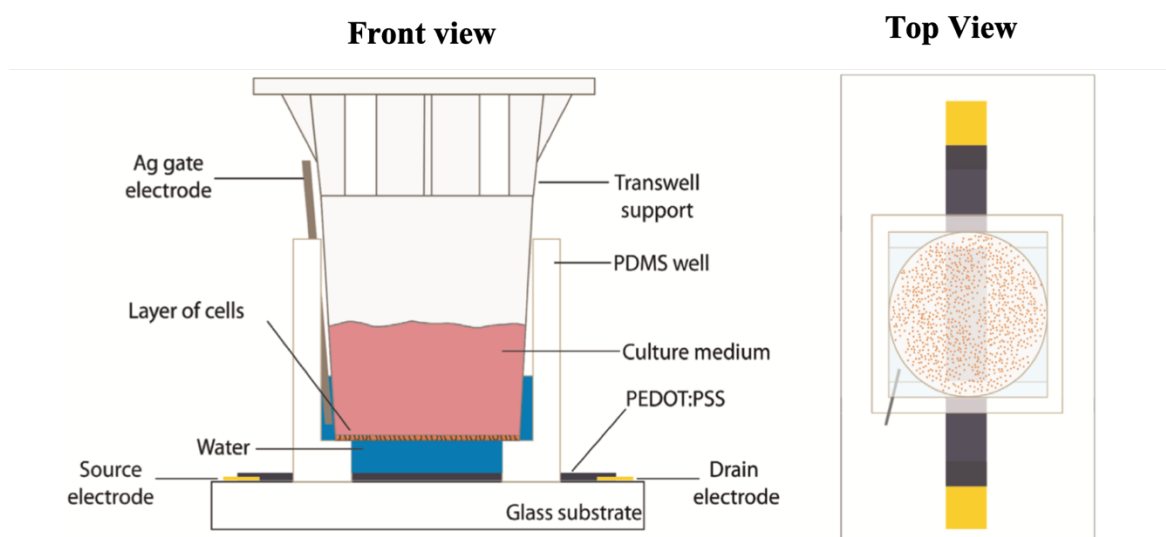


Figure 1.15: Schematic of front and top view of an OECT combined with a Transwell support for cell activity sensing.⁹²

The same device geometry was used by D'Angelo et al ⁹¹ for studying osmotic stress upon osmolarity change into the culture medium.

1.3.2.3 OECTs as Bio-signal recording

The combination of biocompatibility of the device polymeric channels, superior amplifying properties, low voltage of operation and the possibility to manufacture them on a flexible and conformable substrate, makes OECTs ideal for electrophysiology signal monitoring directly on the human body and organs.

Khodagholy et al ⁹³ reported, for the first time the *in vivo* use of an OECT for brain activity recording. PEDOT:PSS has been used as active material and all the essential part of the OECT are deposited on a Parylene-C substrate that conferred flexibility and conformability to the device combined with the device biocompatibility, allow its implantation on rat brain and the recording of epileptiform activities with a LOD higher respect to the commercial electrodes.

Later on, Campana et al ⁹⁴ reported an OECT for monitoring electrocardiography (ECG) directly on the skin. The device has been fabricated on a flexible and resorbable substrate (PLGA) and stick to the skin with the help of an Electrolyte gel that acts as an adhesion promoter and an impedance adapter between the skin and the PEDOT:PSS channel. In this work the human body has been used as a gate electrode and the electrical potential of human body has been used to drive the gate current. The ECG monitored with the OECT has been acquired in a typical clinical configuration and has been compared with the ECG registered with a commercial Ag/AgCl electrodes showing how a comparable signal-to-noise (SNR) could be achieved.

Starting from these results, in 2015 Leleux et al reported the application of OECT devices for ECG, electrooculography (EOG) and electroencephalography (EEG) directly on skin. To register these kinds of signals, a good amplification is needed because these three bio-signal are characterized by small voltage peaks, in the range from mV to few microvolts. To overcome this problem, the authors developed a device that presented a high transconductance, amplification of devices, at zero gate voltage⁹⁵ is used so that only a voltage on the channel is needed. As reported in Fig.1.16a the classical Einthoven triangle set up has been used. ⁹⁶

For ECG recording, the gate electrode was connected to the body with an Ag/AgCl electrode; a second electrode was applied on the skin in the opposite position with respect to the first one and was connected to the source. A V_{ds} of -0.6 V was applied and the signal was acquired.

The same parameters were used for EOG and EEG monitoring upon changing the electrode position. For EOG the electrodes were applied near the eyes (Fig. 1.16b), while for EEG recording the two electrodes were applied on the head (Fig.1.16c).

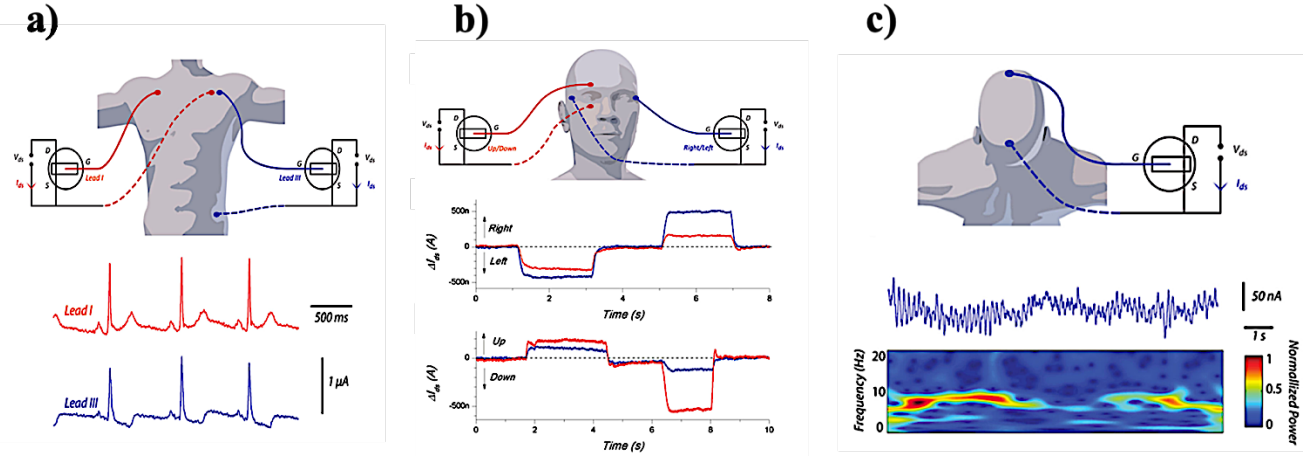


Figure 1.16: In a) Einthoven triangle set up for ECG monitoring. Two devices are used and the two signals are compared. The first device registered the ECG signal between left and right arm; the second is connected between the left arm and left leg. In b) EOG recording set up is reported and two devices are used. The first registered right and left eye movement; the second up and down movement. In c) the EEG set up and the signal are reported.⁹⁷

More recently; Khadagholy et al ⁹⁸ developed a device geometry for *in vivo* monitoring of EEG. The proposed geometry consists of a gate electrode coplanar with respect to the source and drain electrodes position. PEDOT:PSS modified with D-sorbitol has been used as an active layer. PEDOT:PSS doped with D-Sorbitol works as a reservoir of ions. Among the PEDOT:PSS layer and the gate electrode an ion membrane is deposited; in this way a higher transconductance device is achieved (32,30 mS) due to the PEDOT:PSS conductivity enhancement induced by D-Sorbitol doping. The device was mounted directly on the scalp and EEG signals have been registered for 1 hour.

Chapter 2

The present chapter is dedicated to the description of the common printing techniques for flexible and wearable devices with a particular focus on advantages and disadvantages, an important role is attributed to the Aerosol Jet Printer technique with a full description about principle and state-of-the art.

Section 2.1 describes all the printing techniques reported in literature. Section 2.2 describes the working principle of the screen printed procedure and its application for sensing and biosensing. Section 2.3 reported the working principle of the well-known ink-jet principle with its advantages and limits.

Section 2.4 depicted the innovative and prominent aerosol jet printing (AJP) with how this process can overcome the common technique limits with a focus on its applications.

2.1 Printing Technologies

Additive manufacturing and printing electronics over flexible substrates are areas of great interest since give rise to a fantastic design freedom and architectural flexibility while requiring relatively small investment and fabrication costs at least for small and easily customizable productions also in a perspective of large area flexible electronics. On the basis of the ongoing trends, these fast evolving technologies are expected to strongly impact in particular on the on flexible/bendable sensors and conformable electronics fully exploiting polymeric materials as substrates in combination with a large variety of different materials including nano systems and biomaterials. The change of paradigm is related to the advantages that printing processes represent with respect to the traditional and expensive optical lithography. These approaches typically require simpler technological process steps, efficient use of the materials with a reduced material waste, low fabrication costs, simple generation of complex patterning and architectures combined with non-planar surfaces and in some cases compatible with additive 3D and 4D processes ^{99,100} so that these emerging and fast growing technologies are ideally suitable for flexible and wearable device development.

The literature demonstrating the recent developments of the field is growing rapidly including developments on actuators ^{101,102}, light emitting diodes ^{103,104,105}, transistors ^{106,107} and bio-sensors <sup>108–
¹¹⁰</sup> development. The available approaches could be classified, for the sake of simplicity and with

reference in particular to flexible sensors and electronic devices, in the two major classes: contact and non-contact printing ¹¹¹ (Fig.2.1).

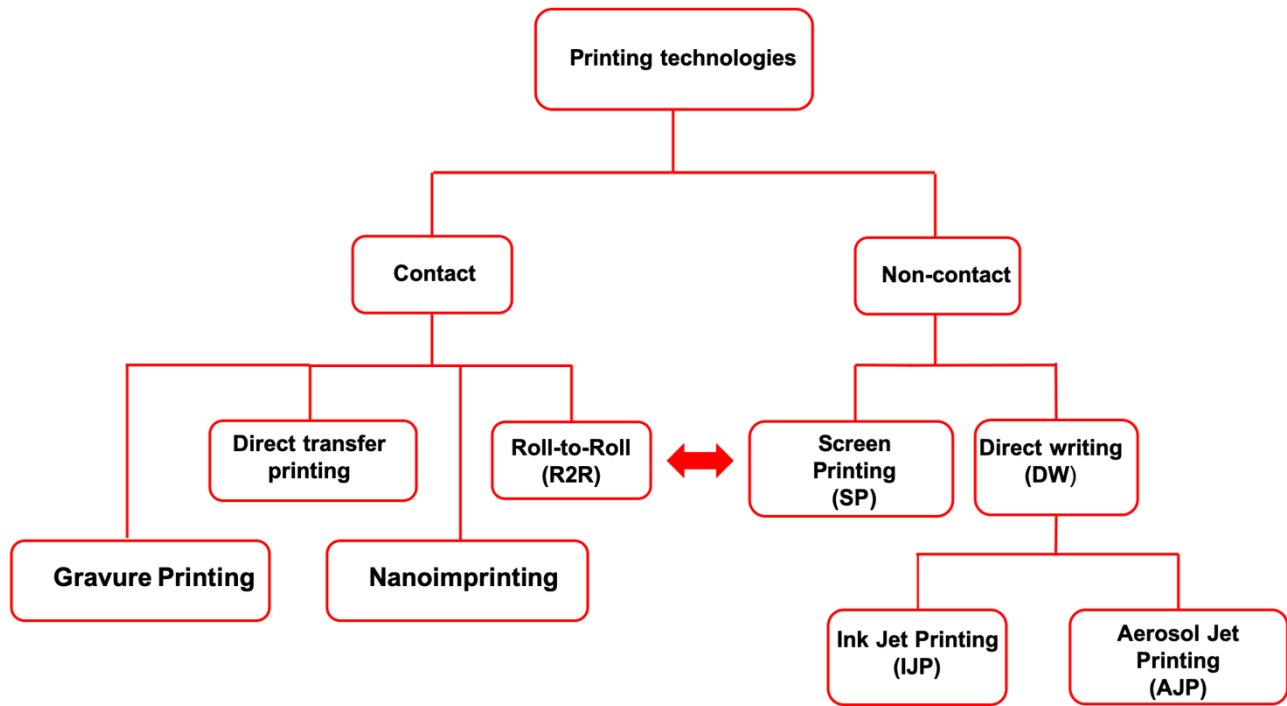


Figure 2.1: Schematic classification of the fundamental printing techniques (Redesigned from ¹¹¹)

The contact mode printings can be considered an already traditional process used since long time in devices fabrication. This family of techniques basically consists in the use of a pre-patterned mask in contact with the substrates (flexible or non-flexible) and the inks arrive on the substrate through them ¹¹². Examples of contact printing are: the well-known and widely spread direct transfer process, ¹¹³ rot-to roll (R2R) techniques, ¹¹⁴ gravure printing ¹¹⁵ and nanoimprinting. ¹¹⁶ Even though all such methods are still fast evolving including new functionalities, in the last decades, increasing efforts have been devoted to the development of new printing techniques. These non-contact printing processes have become more and more appealing because of their simplicity and versatility in terms of materials and architectures, affordability, prototyping speed, high resolution, efficient use of the materials and reduced wastes easy control adjusting a few parameters and the ability to directly fabricate devices on several types of substrates. ^{117,118,119}

2.2 Screen printing techniques

Screen printing is the most matured and widespread technology for printed electronics. It is fast and versatile in comparison with the contact printing techniques. Typically, there are two screen printing setups: flatbed and rotary.¹²⁰

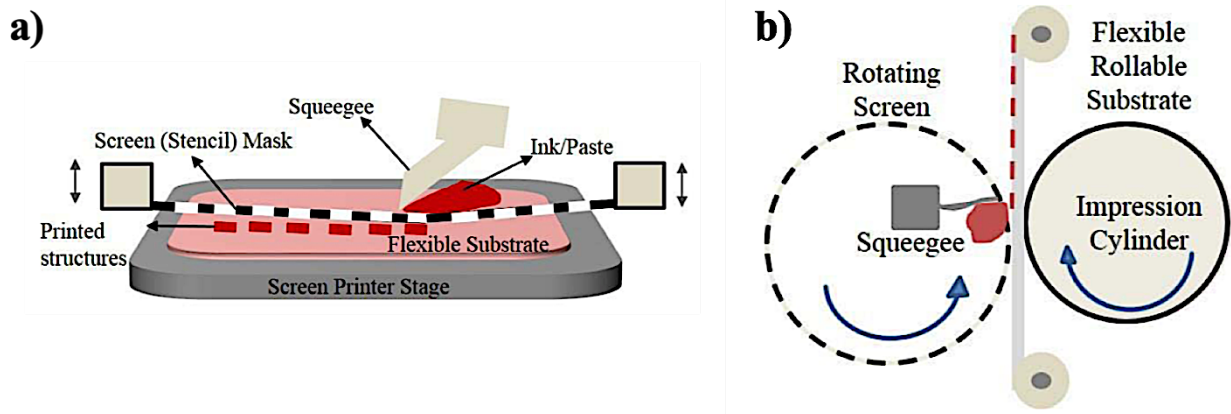


Figure 2.2: Schematic of screen printing major process. In a) the flat bed process is reported; the flexible substrate is on the screen printer stage, over the substrate is imposed the pre-patterned screen and through a squeegee the ink is led to pass through the mask. In b) the rotary set up is shown. The flexible substrate travels between two cylinders: the impression and the rotating screen. The rotating screen is the pre-patterned mask and inside which is positioned the squeegee and the ink reservoir¹¹¹

A screen-printing set up is composed of a screen, a squeegee, a press and the substrate. In the flatbed set up the flexible substrate is positioned on the screen-printing plate and the patterning screen allows that the ink is being squeezed through the screen on the flexible substrate. The ink reproduces hence the pattern of the screen onto the flexible substrate. The flatbed set up is typically a laboratory instrument, to scale up to an industrial level a revolving set up has been developed.

In the rotary set up there are two rotating cylinders on the opposite sides of the flexible substrate, one of which is patterned with the desired design and contains the squeegee and the inks. Relatively high speed is achieved by the rotating screen in comparison with the flatbed, with a major drawback that the rotary screens are very expensive and difficult to clean.

The typical width resolutions achievable with screen printing technique range between 50 to 100 μm with a few microns in thickness.¹²¹ The technique resolution depends on several parameters: substrate surface tension, speed, ink viscosity, angle and geometry of the pattern.^{122,111}

Screen printing techniques have been widely used for fabricating a number of different types of sensors and electronic devices. For example, in 1997 has been reported the first plastic transistor¹²³ fabricated with the screen printing techniques where all the essential components are printed on a plastic substrate.

Only quite recently, in 2017, organic electrochemical transistor for selective ascorbic acid sensing³⁷ has been reported where flatbed printing process has used to fabricate the devices on polyester film and all the OECT components where printed. A Limit of Detection (LOD) around 80 μM is achieved.

2.3 Ink-jet printing

Ink-jet printing (IJP) is widely recognized as an efficient method for direct deposition (writing) of a functional material on a substrate (flexible or non-flexible) in a predesigned pattern. The technique is characterized by a simple low-cost process, with a low waste of materials and well suited for large area fabrication. IJP is a direct writing (DW) technique where an ink, made of functional materials dissolved or suspended in organic solvents, is directly printed on a substrate through a nozzle.¹¹⁷

In the literature a series of different mechanisms to actuate/control the ink-jet nozzle are reported, among which the most prominent are the thermal^{124,125}, piezoelectric^{126,127} and electrohydrodynamic inkjet systems.^{128,129}

In the thermal system the actuator to generate the ink droplets exploits the thermal expansion of the ink to generate a steady ink jet.¹²⁵

The piezoelectric (PZT) system consist of a PZT actuator that generates the ink jet by an acoustic wave.¹²⁶ In the electrohydrodynamic system, the solution is ejected by generating an electrical field between the nozzle and the substrate that works as counter electrode. The printing is achieved typically in two different modes: continuous (CIJ) and Drop-on-Demand (DOD).¹³⁰

CIJ works by continuously ejecting drops from the reservoir at a constant frequency. After the nozzle exit a shutter system can stop the printing without turning off the flow. In the DOD mode, a signal is sent to the transducer only when the drop is needed.

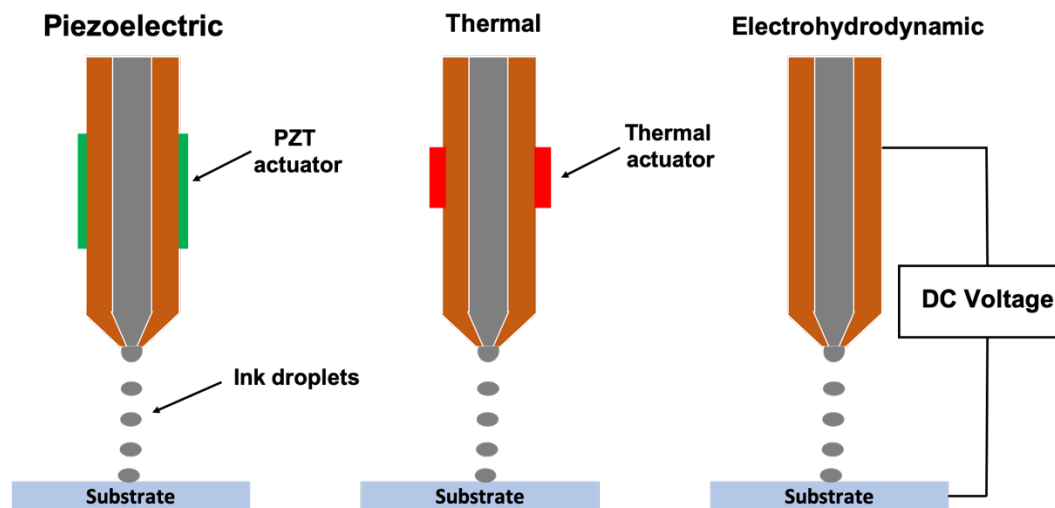


Figure 2.3 Schematics of the principal IJP techniques. In a) a PZT actuator is used to produce the ink droplets through the application of an appropriate acoustic wave. In b) a thermal actuator is used. In this set up temperature expansion of ink is used to generate droplets. In c) an DC voltage is applied between the nozzle and the substrate to produce a charging droplets migration.
(Redesigned from ¹³¹)

The typical droplet diameter for CIJ is around 100 μm while is typically 20-50 μm for a DOD. A weakness of the CIJ printing is that some unwanted ink is used and hence wasted during the printing process¹³² while in the DOD process only individual drops are produced and printed on the substrate, so that the process became more economic because ink consumption decrease.

A typical resolution for an IJP process is $\geq 20 \mu\text{m}$ and it depends on several parameters: surface energy tension, ink viscosity, the voltage applied and stage speed. ¹³³

Very important and in some cases critical is the role of the inks, their composition and characteristics that determines both the printing and post processing processes. Typically, the desired material is a solute dissolved or suspended in a solvent or a mix of solvents with a high evaporation rate. The volatility of the solvents is quite important to achieve well defined features. A critical point is the formation of the so-called “coffee ring effect” (CRE) that limits often the resolution achievable and performances of the printed lines. Coffee ring effect is generated by a non-uniform distribution of the ink droplets from the center to the edges. CRE affected features present a thin center surrounded by thick edges forming a structure like “ring”. Several methods have been proposed to minimize this effect by adding surfactant ¹³⁴ and compositional solvents such as Ethylene Glycol or Butanol. ¹³⁵

With highly volatile solvents the ink deposited on the substrate dries up in a few seconds and the coffee ring effect (CRE) decreases. CRE is depending on the solute concentration in the ink, the

higher is the solid fraction of the material the lower is the coffee ring effect.¹³² Other parameters that determine the IJP resolution and the printability is the ink viscosity. The typical range for IJP process is around 10-20 cP, with this range is possible avoid the nozzle clogging.¹³⁶

IJP is limited by ink viscosity and this parameter delimits the kind of materials and features that is possible to print with this process. IJP techniques could be considered the most used and performing printing processes to develop flexible devices such as LED, sensors, OTFTs and Photonic devices. Among the examples most relevant for the present work, is worth mentioning: Mannerbro et al¹³⁷ have demonstrated a printed organic electrochemical transistor (OECT) where both the channel and the electrolyte are printed from PEDOT:PSS and Electrolyte solutions respectively while already in 2014 an all- printed OTFT is reported¹¹⁰ where metal contacts and active material are printed and then functionalized with antibodies for label-free detection of protein.

Ink-Jet Printing technique has been use also for developing passive electrical components such as inductors, capacitors and resistor¹³⁸ that render makeable developed full printed electronic circuits on large scale with an high features definition and combine this with active components (such as OFET, OLED and OECT) with few working steps and low consumption of materials, impossible to achieve with other common printing techniques such as screen printing.

Also, IJP renders possible deposited material on non-flexible and fragile substrate, i.e. optical fiber tips. Cox et al¹³⁹ demonstrates the possibility to print a microlenses array of thermoplastic polymer on an optical fiber tip with a dimension of features around 70 μm diameters as reported in Fig. 2.4 with the aim to collimate length on the tip of fiber.

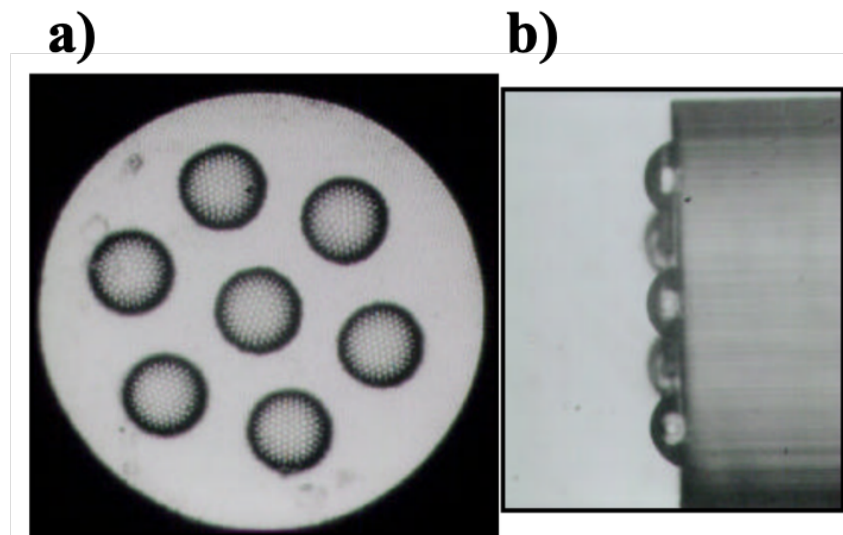


Figure 2.4: a) top view of the single optical fiber with the printed microlenses. In b) the side view.¹³⁹

The advantages and potentialities of the IJP technique has been richly described in literature compare to the common printing techniques. The problem is that this technique presents several constrains such as the narrow range of viscosity printable material (10-20 cP) and a high features definition ($> 30 \mu\text{m}$) that make this technique not the prominent for bio signal monitoring.¹⁴⁰ For this reason, we decide to developed our devices with the aerosol jet printing (AJP) which increase the range printable materials and make possible an in-line devices fabrication with high features definition on 3D substrates thanks to its high working distance (3-5 mm).

2.4 Aerosol-Jet Printing

Aerosol Jet Printing (AJP) represents a promising evolution of IJP enabling progress in terms control of the writing, increased type of materials and definition of fine features with a resolution achievable of about $8 \mu\text{m}$ ¹⁴¹ that could be enhanced up to $5 \mu\text{m}$ by “on flight” printing.¹⁴² Tab.1 compares the major parameters between IJP and AJP.

Non-contact process	IJP	AJP
Printing Priniple	Electrostatic	Aerodynamic
Nozzle diamter (μm)	10-50	100-300
Working Distance (mm)	1	3-5
Ink Viscosity (cP)	10-20	1-1000
Dynamic accuracy (μm)	N/A	± 6
Line thickness (nm)	5 - 500	50* - 5000
Line width (μm)	>30	5 - 200
Particle diameter (μm)	<100	10 - 700

*Thickness achive for PEDOT:PSS in our experimets

Table 2.1 : Major operating parameters comparing IJP and AJP¹⁴⁰

The AJP is a direct, non-contact, mask-less printing process that deposit an ink on a substrate in the form of an aerosol mist.

The aerosol is an aerodynamic focusing spray composed of a mix of ink particles with different size, typically $1-5 \mu\text{m}$,^{141,143} surrounded and transported by a nitrogen gas flow, carrier gas flow (CG).

The working principle of the AJP process can be schematized into three fundamental steps: aerosol formation, aerosol transport and aerosol deposition. Fig. 2.5 shows the schematic representation of an AJP printing set up composed of a system that produces the aerosol mist, the so-called atomizer system, the transport part composed by a pipe line, mist tubes, and a printed system composed by a ceramic nozzle and a deposition head (DH) where the aerosol mist is confined by a second gas flow, sheath gas flow (ShG), that creates a ‘confining shirt’.

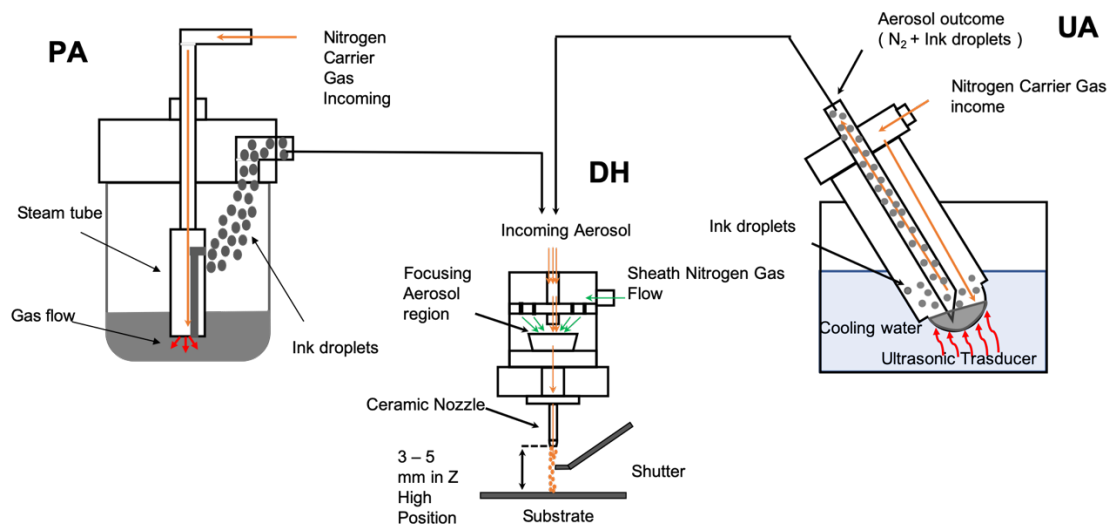


Figure 2.5: Schematics of the Aerosol Jet Printing process. a) Atomization from a liquid ink with Pneumatic (PA) or Ultrasonic (UA) atomizer, b) delivery of the material toward the deposition head through a carrier gas stream, c) sheath gas focusing and d) deposition on the underneath substrate

The aerosol can be formed with two different atomizer systems, pneumatic (PA) and ultrasonic (UA) atomizer, making feasible to print inks with viscosities from 10 to 1000 cPs and 1 to 10 cPs respectively¹⁴⁴. The PA is composed by a plastic jar, the ink reservoir, and a metallic cylinder. High pressure gas flow is applied onto the ink to generate the aerosol.

The aerosol mist is collected in the mist tube and flows into a filter, virtual impactor, that selected and removed all small droplets inside the aerosol. After the filter, the mist is delivered at the deposition head (DH).

In the UA, an ultrasonic wave is exploited to create droplets of inks through a surface acoustic wave. Heavier droplets recombine in the liquid phase, while lighter droplets can be captured by the carrier gas flow and directed toward the deposition head, through a delivery tube (the mist tube).

After being generated, the mist reaches the printing head. As reported in Fig. 2.6, is composed by a focusing chamber where two gas flows, carrier gas and sheath gas, are combined in a coaxial configuration. The mist, surrounded by the coaxial gas flow, gets aerodynamically focused in the ceramic nozzle, 100-300 μm diameter, and then reaches the substrate.

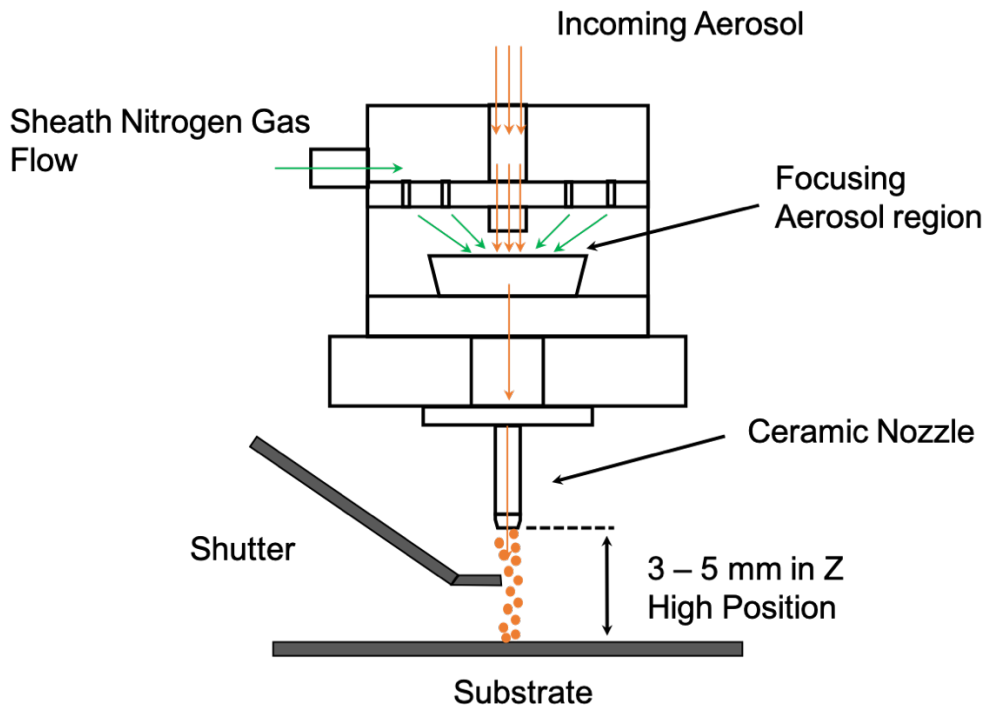


Figure 2.6: Schematic representation of the Aerosol jet Printer deposition head. After the atomization process the mist and the sheath gas arrive in the focusing aerosol region. The sheath gas confines the aerosol and through the nozzle arrives on the substrate depositing the material (Redesigned from ¹⁴⁵).

The substrate is mounted at 3-5 mm from the nozzle exit, on a motorized and heatable x-y stage that is used to define and design the wanted pattern (CAD pattern) by moving the substrate with an accuracy of about 6 μm .¹⁴³

As reported in Tab.2.2, AJP can process several kind of materials (the list grows rapidly with the increasing number of applications) with a wide range of viscosity ranging from 1 to 1000 cP, defining line widths from <8 to 5000 μm .¹⁴⁶

AJP Materials			
Conductive materials	Oxides	Dielectrics	Bio-materials
Gold ^{147,148}	Al ₂ O ₃ ¹⁴⁹	SU-8 ³⁹	DNA ¹⁵⁰
Silver ^{151–153}	RuO ₂ ¹⁵⁴	PVP ¹⁵⁵	Protein ¹⁵⁰
PEDOT-PSS ^{39,156,157}	ZnO ¹⁰⁶	NEA 121 ¹⁵⁸	Enzyme ¹⁵⁰
P3HT ¹⁵⁶		NOA 81 ¹⁵⁹	Cell ¹⁶⁰
CNT ^{159,161}			

Table 2.2: Summary of materials printed by AJP procedure

To achieve a good line definition of the deposited material and a controllable/reliable process, the control of several parameters is quite critical. The process parameters for the AJP can be summarized as follows:

- **Atomizer Flow (sccm):** this parameter controls the carrier gas flow rate present in atomizer systems
- **Sheath Gas Flow (sccm):** this parameter controlling the sheath gas flow rate determines the degree of confinement and collimation of the mist in the DH.
- **Frequency Ratio (FR) (a.d.):** this parameter is defined as the ratio between the atomizer flow rate and sheath gas flow rate and determines the regime of confinement of aerosol is in a coaxial gas ‘shirt’

$$FR = \frac{ShG}{CG} \quad \text{Eq. 2.1}$$

- **Exhaust Gas Flow (sccm):** this parameter controls the flow rate used to filtered the aerosol mist after pneumatic atomizer exit by letting part of the flux with the largest particles to be eliminated from the flux.
- **Platen Temperature (°C):** it is the platen temperature the regulation of which determines the evaporation rate of the solvent of the ink during and/or after the deposition and hence controls

the drying process on the substrate. This parameter is quite relevant to control the final physical-chemical characteristics of the material deposited.

- **Nozzle diameters (μm):** this is the inner diameter of the ceramic nozzle tip that focuses the aerosol mist on the substrate. The definition of the features deposited and the overall performance of the deposition is depending on this parameter in conjunction with the type of material, the solvents used, FR etc.
- **Working Distance (mm):** this is the perpendicular distance between the nozzle exit and substrate. Typically, during the printing process, this distance is maintained constant (5mm) to achieve the most repeatability process.
- **Stage speed (mm/s):** this parameter controls the stage speed, in other word the substrate speed, and determine the line thickness. The higher is the stage speed the lower is the line thickness.

Several conditions play a crucial role in the printing process as reported in Fig.2.7

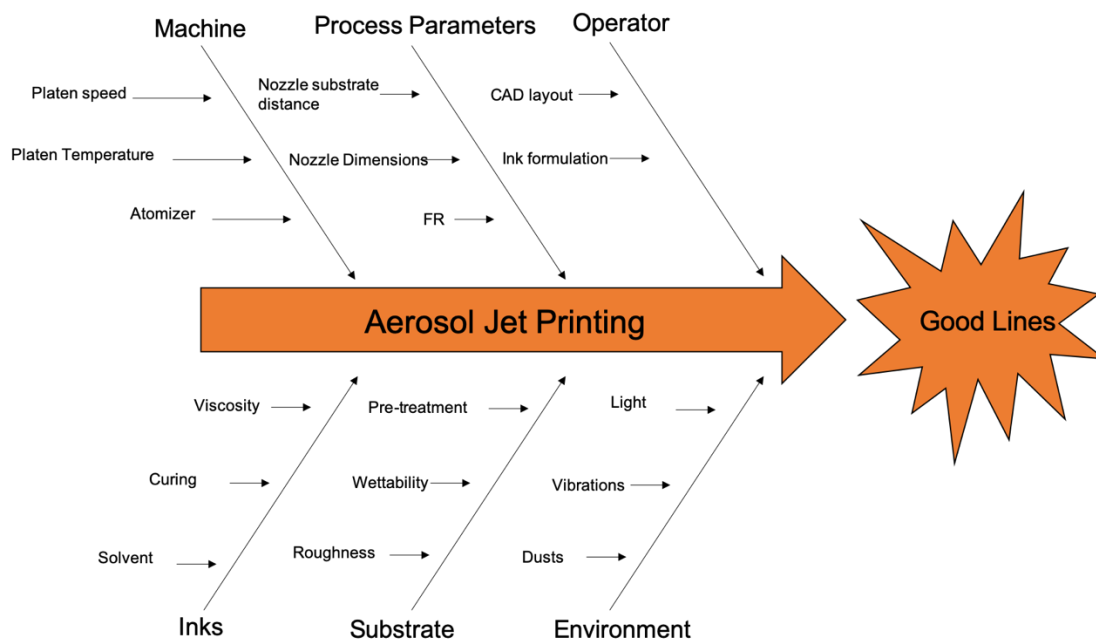


Figure 2.7: Summary of relevant AJP parameters (Redesigned from ¹⁶²)

The final performance of the AJP could be achieved for each specific combination of ink, substrate etc, by finally tuning the different parameters involved. Anyhow large efforts have been devoted to define a compromise between these parameters and to search for an operating window. All the early studies have been carried out on inks of silver nanoparticles. Goth et al ¹⁶³, for the first time, reported a preliminary analysis on the dependence of printed line features from the printing parameters. Later on, in 2014, a more systematic study onto the influence of these parameters on the morphological and electrical properties of the silver printed lines has been published demonstrating that a key parameter should be considered the already mentioned FR that controls the aspect ratio of the printed lines. ¹⁵²

The importance of FR is further endorsed by an analytical study by Binder et al ¹⁶⁴. More recently, Smith et al. ¹⁶⁵ extended the experiment introducing the effect of the substrate temperature. In all of these studies great attention is devoted to minimizing the overspray (OS) effect.

OS is a printing artefact due to droplets that have an insufficient inertia to be impacted close the central line of the aerosol flow ¹⁶⁶and hence producing a loose definition of the printed line due to the deposition of droplets of ink out of the line itself. If the density and velocity of the droplets are the same, these inertial difference will be attributed to a different dimension of the droplets ¹⁵⁸ that are not focused with the same efficiency by the deposition head.

OS issues are particularly problematic in miniaturizing process where they could cause possible cross-talking and undesirable interconnection that may arise in close and adjacent structures. OS effects remain a challenging open issue. Recently, several analytical models have been proposed aiming at improve the understanding of the printing mechanism and to achieve robust print recipes.

^{143,158,167}

In this framework Chen et al. ¹⁵⁸ report on a computational fluid dynamic (CFD) model of the CG, that is confined by a coaxial gas flow, the SHF. The model aims at understanding the principles that control the OS in a typical AJP system. A dependence between OS and SHF (CHF is maintained constant in the computational) is reported. The authors show that the OS decreases as the SHF increases up to a value of SHF at which there is the onset of turbulence at the nozzle exit and hence there will be an increase of OS. The abundance of small diameter droplets ($d = 1-5 \mu\text{m}$) is also demonstrated to play a role on the OS, due to the low confinement of SHF at the nozzle exit.

Secor et al ¹⁴³ have demonstrated the importance of machine geometry and transport losses in ill-defined lines. In fact, the loss of solvent of the ink's droplets due to the carrier gas during the trip in the mist tube, can cause that they will arrive in the DH with a low solvent content or totally dry hence contributing to the OS along the line edges.

2.4.1 AJP applications

As already mentioned, the AJP technique is a relatively simple process, characterized by low cost, low waste of materials and great versatility including adaptability to large area fabrication. These characteristics, combined with a wide range of processable materials, a max resolution $<8\text{ }\mu\text{m}$ and the ability to print on non-conventional materials make AJP a very promising technique in microelectronics applications. Aerosol deposition has been successfully used to print inorganic materials for the manufacturing of antennae¹⁶⁸, extended to a great variety of organic and bio-organic systems to fabricate resistors and capacitors³⁹, interconnection¹⁶⁹, ZnO-based photodetector¹⁷⁰, Organic Field Effect Transistors^{107,156} and bio-molecular materials for biosensing.¹⁵⁰

The first research publications featuring AJP technique became to emerge around 2001-2002 when Marquez et al¹⁶⁰ directly printed several bio-materials (cells, enzymes and proteins) directly on a culture media for the first time by an aerosol jet printer. After deposition, viability studies demonstrated the good viability of printed bio-materials.

Starting from this milestone, Grunwald et al¹⁷¹ demonstrated the ability to print DNA strands and enzymes without any kind of damages in their biological activity. An agarose gel electrophoresis was used to investigate the DNA integrity before, during and after the printing process. Both pneumatic and ultrasonic atomizer were used showing that only the pneumatic atomizer preserves DNA integrity as shown in Fig.2.8

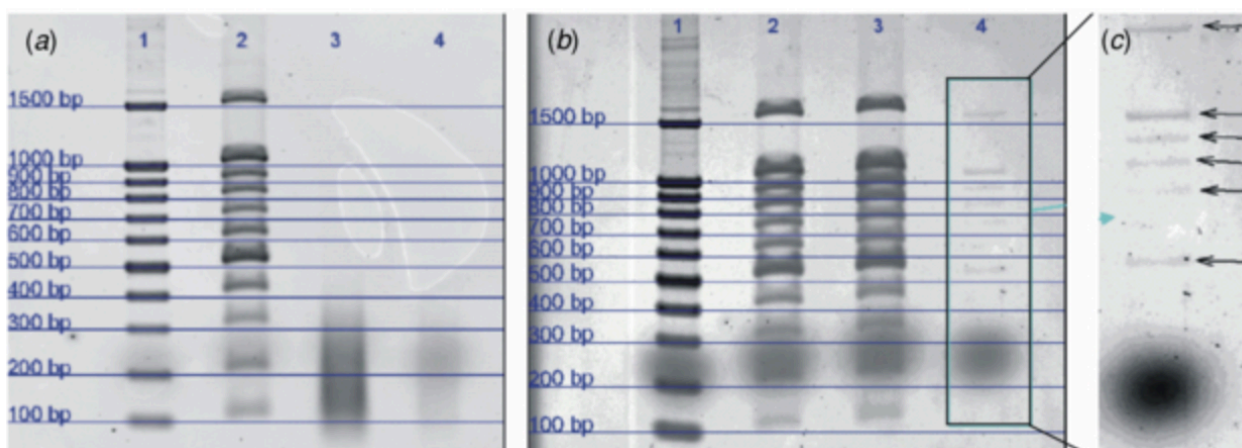


Figure 2.8: Comparison of printed DNA by UA and PA atomizers. In a) electrophoretic analysis for DNA printed with UA is reported. Before the ultrasonic wave application, the DNA is stable after printing it loses his biological integrity. In b) PA is used and it is evident that DNA maintain his biological integrity¹⁵⁰ through the printing process. c) shows the magnification of the DNA spot after deposition.

Marquez and Grunwald demonstrate the suitability of AJP for printing biomolecules paving the way to a new era for microarray bio-fabrication.

Applications of the AJP to print conductive traces designed to interconnect two or more electronic components are widely reported in literature. Because of their simplicity these components have been the primary structure developed to explore the huge potential of this technique in microelectronics applications.

The first application of the AJP to print interconnects is reported in an article of 2007 where highly conductive traces for silicon solar cell metallization were made by a silver nanoparticle ink¹⁷².

Padovani et al ¹⁶⁹ deposited silver ink traces (35 μm) on a glass substrate to interconnect a LED array on a transparent display.

The z-axis easy positioning of the DH has allowed the production of vertical interconnection. For example Zhan et al ¹⁷³ reached a good coverage of trapezoidal and reverse trapezoidal holes to interconnect the top and bottom silicon parts of MEMS device as shown in Fig 2.9b.

One of the most advantages of AJP is its ability to produce fine features with demonstrated line width of 10 μm reported by Cai et al ¹⁷⁴. The ability to print fine lines is used by Kopola et al ¹⁷⁵ to improve device performances by decreasing the width of tracks in the current collection grid of a reverse ITO solar cell.

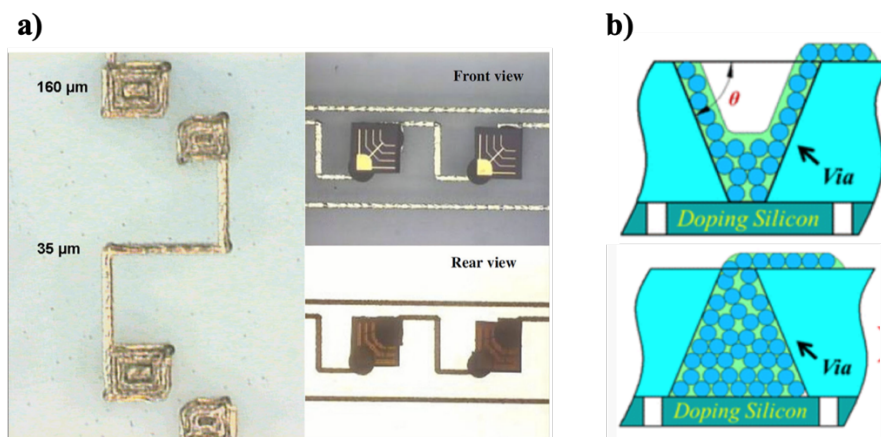


Figure 2.9: In a) the silver printing interconnections are reported with a 35 μm width for the line and 160 μm for the pad. The ability of AJP to produce fine structure is reported. Also, in a) is reported the top view and the rear view of the silver backbone in contact with the LED devices ¹⁶⁹. In b) an example of 3D structure covered by AJP is reported. The simple way with trapezoidal structure and the difficult way reverse trapezoidal structure are shown ¹⁷³.

As already mentioned, another appealing feature of AJP technique is the ability to print a wide range of materials. Gupta et al ³⁹ used this technique for resistor and capacitors productions. Silver ink is printed on a Kapton substrate and several couples of silver pads with different distances are prepared. After silver deposition, PEDOT:PSS is printed between the two contacts. In this way an array of resistors is obtained. The resistance increase with the length of the PEDOT:PSS channel. In the same article is reported the first full-printed capacitor. Silver ink traces are printed along a SU-8 dielectric medium. A linear increase of capacitance is obtained with the increase of the overlap between the printed silver lines and the dielectric medium.

In the 2008 Cho et al ¹⁴⁷ reported a gel organic thin film transistor (GEL-OTFT) where all the essential part of the devices were AJP printed over a Kapton substrate. They showed, for the first time, the performance achievable with a printed ion gel to fabricate devices achieving a switching speeds up to 10 kHz.

Xia et al¹⁷⁶ have published a GEL-OTFT fully-printed operating at a voltage below 2 V where the major key to obtain the low operating voltage was the ion gel used. The device fabrication was quite reliable as demonstrated by the repeatability of the process over the several printed devices including other substrates such as PEN.

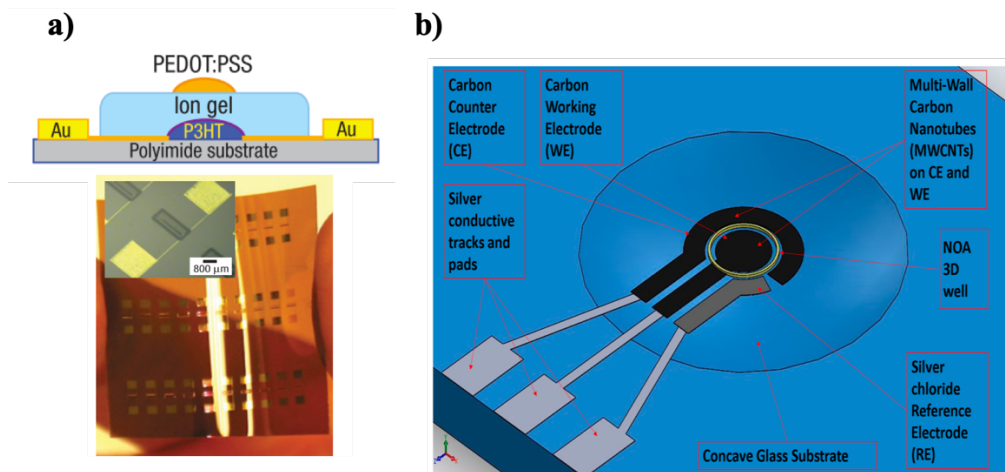


Figure 2.10: a) schematics of the first fully-printed GEL-TFT.¹⁷⁶ All the essential parts of the transistor are made by AJP printing: the source and drain electrodes are printed from an Au ink; for the gate electrode a PEDOT:PSS ink was used while P3HT and Ion gel inks were respectively used to print the channel and the electrolyte. a) shows also the array of devices as printed on Kapton. b) schematic of protein sensor produced by Cantù et al ¹⁵⁹.

Recently, Cantù et al ¹⁵⁹ proposed a full-printed electrochemical sensor for the detection of Interleukin-8 where a silver nanoparticle ink is printed to produce the conductive traces, a carbon ink is used to print both the working (WE) and the counter (CE) electrodes, a silver chloride ink for the reference electrode (RE) and finally NOA 81, a UV-curable passivation polymer ¹³⁶, is printed to create delimiting edges to border liquid samples on the active channel. A PA was used to print all the essential part of the device. They used an Anodic stripping Voltammetry for protein detection achieving a LOD of 0,3 ng/L by coating the WE with multiwall carbon nanotubes (MWCNT).

In summary AJP has already demonstrated to be a viable and quite promising technique for the fabrication of devices integrated on polymeric substrates even though the field is still at the early stage of prototype demonstration. On this basis the present work is aimed at contributing at further developing the method both using different substrates and inks in particular for electrochemical devices on flexible and potentially stretchable substrates.

Chapter 3

The chapter is dedicated to description of the advantages that silk fibroin introduced in the bioelectronic world. Section 3.1 describes the internal structure and the properties of silk fibroin with a particular focus on the strategies developed to tune its mechanical and optical properties. Section 3.2 describes the silk fibroin applications reported in literature; Section 3.2.1 describes the use of silk fibroin as substrate for wearable devices; Section 3.2.2 describes the use of silk fibroin as dielectric layer in organic transistor for sensing and bio sensing; Section 3.2.3 describes the use of silk fibroin as an innovative material in photonics and optical sensing.

3.1 SILK: an ancient material of the future

Silk is a complex biopolymer that is produced by a biosynthesis process in epithelial cells of some lepidoptera larvae such as silkworms, spiders and flies.^{177,178} Silk internal structure, composition and properties differ depending from the larvae that produce it.¹⁷⁹

Silkworm silk from *Domestic Bombyx Mori* is the most characterized and studied one. It has been used for medical sutures (SOLFSILK™, PERMA-HAND™) for decades, and as a textile for centuries. The internal structure of silk fiber consists of a protein core, fibroin, surrounded by a glue-like protein coating, the sericin^{180,181,182} as reported in Figure 3.1

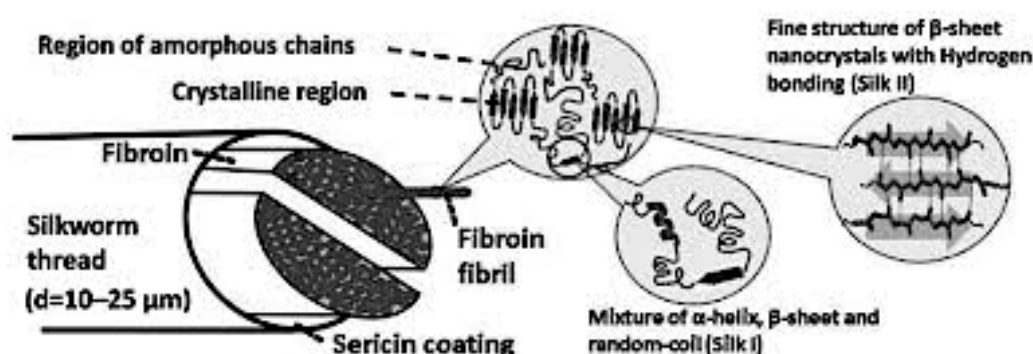


Figure 3.1: Silk fiber internal structure. Silk is composed of two strands of fibroin surrounded by a glue-like cladding. Fibroin is composed by a hierarchical structure composed of a sequence of crystalline and amorphous regions.¹⁸²

Silk Fibroin (SF) is the most important protein of the silk, the properties of which depend on its structure. SF consist of a light (L) chain polypeptide ¹⁸³ and a heavy (H) chain polypeptide ¹⁸⁴, 25 and 335 KDa respectively that are linked together by a single disulfide bond at the C-terminus of the H chains so that an H-L complex is formed.

The superior mechanical properties of SF are H-chains dependent, because of its ability to generate β -sheet structure. ¹⁸⁵ H chain is composed of sequences of several kinds of amino acidic groups in different proportions. A typical composition of an H chain in silkworm SF is the following: Glycine (G) ~ 43-46 %, Alanine (A) ~ 25-30 % and Serine (S) ~ 12% and some Tyrosine (T) ~5% . ¹⁸⁶ These sequences and proportions depend on several parameters: ambient condition ¹⁸⁷, worm species ^{188–190} and feeds ^{191,192}. Fig. 3.2 shows the H chains chemical structure and composition.

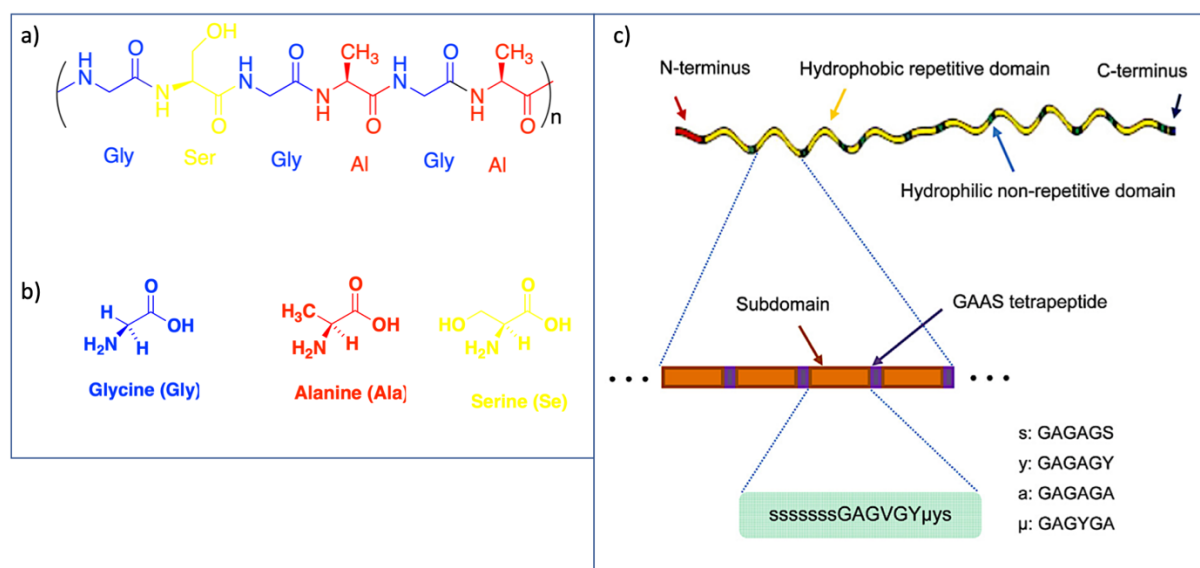


Figure 3.2: a) Silk fibroin is a long polypeptide chain composed by an alternative sequence of amino acid groups. b) Glycine (G) ~ 43-46 %, Alanine (A) ~ 25-30 % and Serine (S) ~ 12%. The fibroin strand is composed of two polypeptide chains with different weight L and H-chain. The H-chain is the one most responsible for fibroin mechanical properties. In c) a schematic of H-chain structure is reported. ¹⁹²

The H chain is divided into 12 hydrophobic domains, containing amino acids in repetitive sequences, alternating to 11 hydrophilic domains, i.e. amino acids in non-repetitive sequences. The repetitive domains (composed of glycine, alanine, serine and tyrosine) can organize themselves in crystalline regions, the so called β -sheet crystallites, by intermolecular forces, including hydrogen, van der Waals

and electrostatic bonds.^{184,193} According to Zuo's model¹⁹⁴, each hydrophobic region is composed of six subdomains that can form β -sheet crystallite presenting a double-layer solenoid structure, as reported in Fig. 3.3

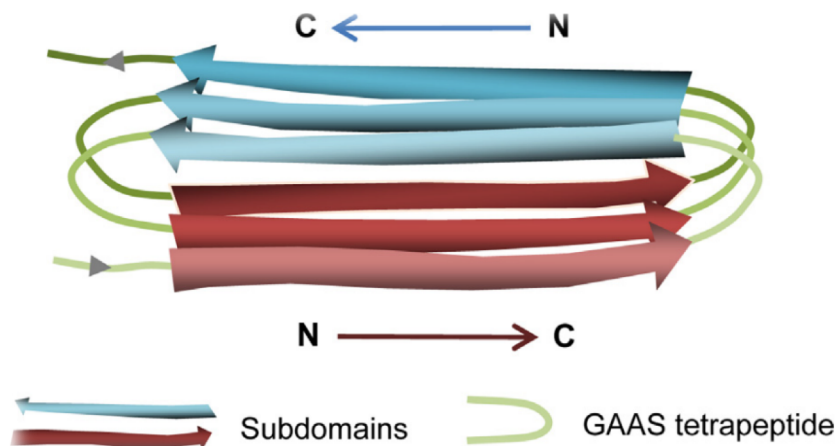


Figure 3.3: Schematics of Double-layer solenoid structure proposed by Zhou et al in which H-chains subdomains are packaged to form β -sheets crystallized.¹⁹²

On the other hand, the hydrophilic regions tend to form some amorphous regions, characterized by random coil structure of the chain.

One of the most important silk characteristics making it intensely studied in the last decades is superior mechanical properties. In case of native silkworm SF, 300- 700 MPa tensile strength can be observed^{195–197} depending on β -sheet amounts^{198,199}, size^{200,201,202} and orientation along the polypeptide backbone axis.²⁰³ Karen et al²⁰² proposed a mathematical model where an optimal crystallites dimension, around a few nanometers, is needed for high strength and flexibility.

Mechanical properties, combined with biodegradability, biocompatibility and easy processability make SF one of the most promising materials for interfacing with biological systems, hence for bioelectronics applications.

The explosion of SF can be traced back to a state of the art work by Rockwood et al²⁰⁴, where a detailed protocol to extract fibroin protein and dissolve it in water is reported. This procedure, in fact, paved the way to the development of several kinds of structures¹⁸² that increased the number of allowed SF applications. Regenerated SF can generally be classified into four morphological types (Fig. 3.4): sponges²⁰⁵, mats²⁰⁶, hydrogel²⁰⁷ and films.^{208–214}

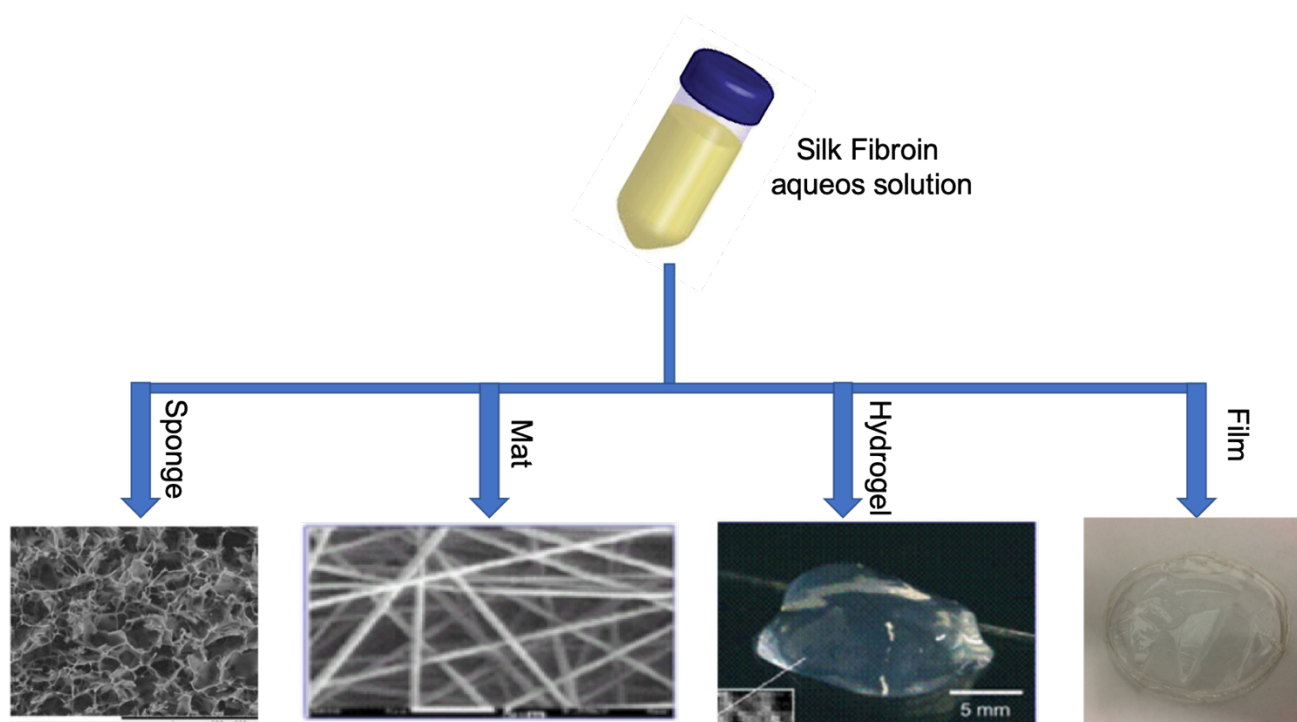


Figure 3.4: Schematics of the four major morphologies that is possible to prepare from the silk regenerated aqueous solution: sponge²¹⁵, mat²¹⁶, hydrogel²⁰⁷, and film.

Regenerated SF exhibits mediocre mechanical properties compared to those of untreated ones. The detriment of mechanical properties is due to protein degumming and dissolution process inducing chains hydrolysis. This type of SF, referred to as Silk I, is characterized by a low crystallinity index. The low crystallinity is determined by the predominance of a random coil and α -helix structure.^{194,192, 217,218}

To overcome these issues, three methods have been developed to enhance SF crystallinity: chemical, thermal and water-annealing methods. All these methods induce a transition from Silk I to Silk II, and hence a structure presence of β -sheets.

The chemical method consists in dipping SF in methanol or ethanol aqueous solutions, at various dipping times inducing the formation of β -sheet crystallites by combining the dehydration effect of methanol with the swelling effects of water. A direct and quick transition from Silk I to Silk II is achieved, as reported in Fig. 3.5, with a good degree of control and repeatably.²¹⁹

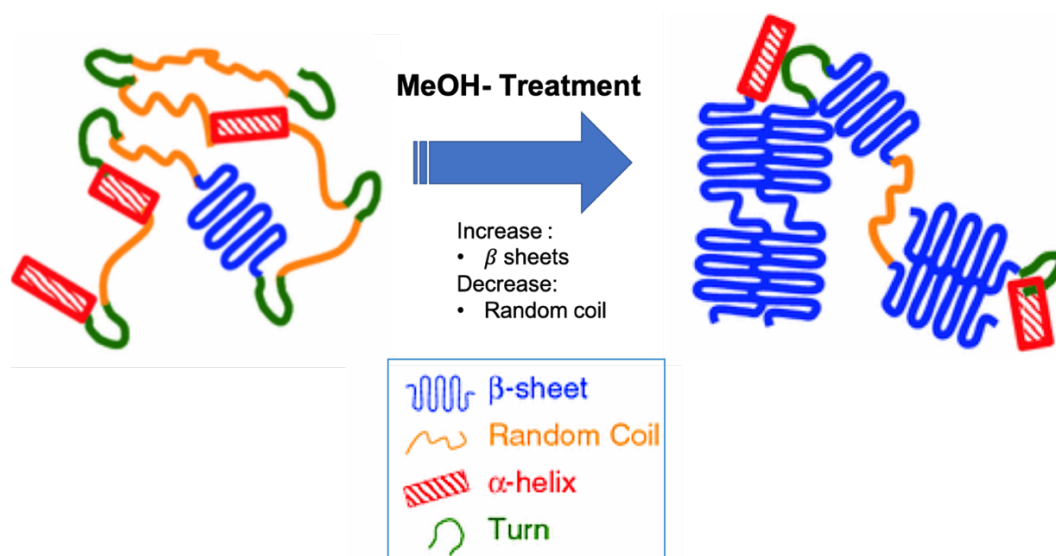


Figure 3.5: Schematics of methanol method. Exposing regenerated fibroin to methanol the β -sheet crystallization increases and the random coil portion decreases. A direct transformation of silk I into silk II is then achieved. (Redesigned by ²²⁰)

The thermal treatment consists in exposing SF to a higher enough temperature (larger than 100°C) for a few minutes. In this way, after the total removal of the water inside a rearrangement of the internal structure occurs that induces the formation of a β -sheet crystallization. When the temperature exceeds the T_g (177 °C) a plasticization of the amorphous region (random coil) is achieved.²²¹ With this procedure it is possible to change the crystallinity index of the material over a wide range and with a high degree of control.

The last procedure mentioned above, the water-annealing treatment²²², consists in exposing SF to the combined effects of water vapor and increased environmental temperature. This procedure gives a much better controlled transition by simply the duration of the process since the transition occurs over a long timescales (hours). A new protein structure is predominant during this treatment, i.e. the so called α -helix (Fig.3.6)

An enhancement of water insolubility and strength textile are associated to the increasing content of this structure in Silk. It is hence desirable to obtain an intermediate fibroin structure where the best flexibility and stretchability of Silk I are combined to the best water insolubility and strength textile of Silk II.

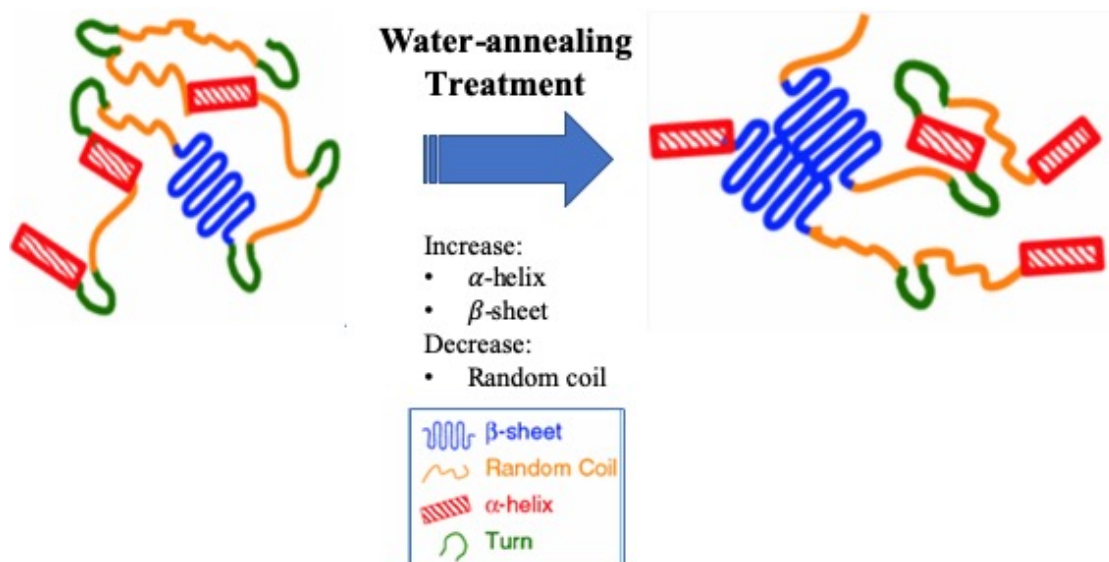


Figure 3.6: Schematics of the “water-annealing treatment”. With respect to the chemical treatment β -sheet crystallize formation occurs more slowly and controllably. Increasing the temperature the fibroin internal structure changes and a slow transition from random coil to α -helix and finally to β -sheet is achieved (Redesigned by ²²⁰).

3.2 Applications of Silk Fibroin films

The possibility to tune silk mechanical properties by acting on the amount of β -sheets, combined to a set of noticeably properties, such as programmable biodegradability, easy processability, biocompatibility, optical transparency, electrical insulator, natural abundance, ultra-smooth and thermal stability, make SF materials, especially in their thin film form, the most promising natural and “green” material for organic electronics and bioelectronics. SF applications are now spread over quite different fields, such as drug delivery, biosensing, fabrication of implantable scaffolds, tissue engineering and, finally, in optical applications.^{223–225}

3.2.1 Fibroin as a substrate for implantable and flexible electronics

Fibroin has already been widely used in implantable devices as an efficient tool to transfer active electronic components onto the surface of organs with the aim of achieving conformal contacts and desirable functionalities. To integrate electronic components on SF films, the typical procedure used is the transfer printing, where a low adhesion substrate as Polydimetisiloxane (PDMS) mold is used to transfer the metallic connections and, more in general, electronic parts on a free standing SF film.

In 2009, one of the first flexible devices made by implementing nMOS-TFTs on a free standing SF film, was reported.²²⁶ This device has shown a good mechanical flexibility, with a curvature radius around 5 mm, without any kind of adhesive failure and cracks in the conductive components. The nMOS-TFT response has been tested before and after silk dissolution, that is used as a sacrificial substrate to deposit the transistor directly on the desired site. The reported transfer and output curves show a good retention of electron mobility and threshold voltage upon fibroin dissolution (electron mobility changed from $500 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ to $400 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, while threshold voltage changed from 0,2 V to 0,5 V). The flexible device is implanted as a whole in an animal model to estimate inflammatory diseases. After two weeks the implanted device showed only a partial silk film degradation and no inflammatory response in tissues around the implant was found.²²⁷

In addition, silk fibroin can be used as a tool for achieving a conformal contact between electronics and curved surfaces such as brain, muscles and skin, being the conformational character important to carry out a precise bio-signals monitoring.

A conformable bio integrated device has been developed by transferring an electrode array, embedded on a thin polyamide film ($2,5 \text{ }\mu\text{m}$), on freestanding silk fibroin.²²⁸ After fibroin dissolution, the electrode array was conformably covering a feline brain surface. Three kinds of devices have been demonstrated, the first one on a $75 \text{ }\mu\text{m}$ PI film substrate, the second and the third on $25 \text{ }\mu\text{m}$ and $2,5 \text{ }\mu\text{m}$ PI films, respectively. Bio-signal acquisition upon implantation has shown that the best performing device is the third one, due to a because better conformable coverage obtained in this case.

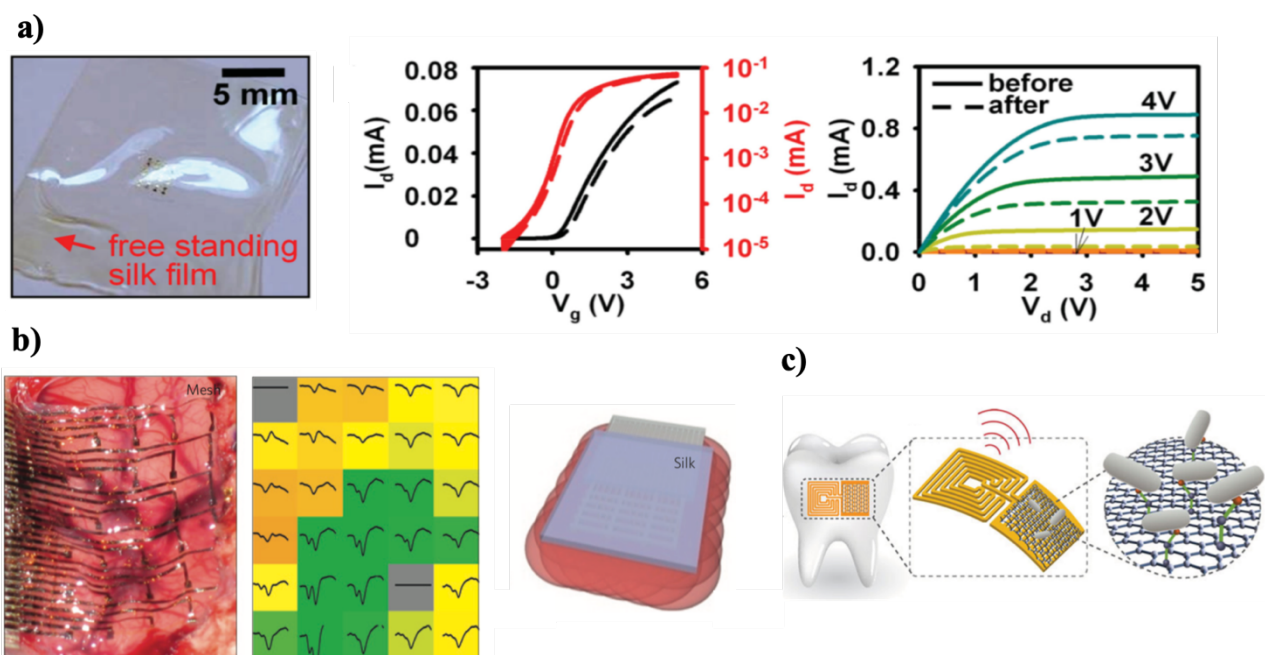


Figure 3.7: a) Ultrathin device on a free standing silk film. The transfer and the output curves are shown in the middle and on right respectively. Transfer and output curves registered both before (solid line) and after (dot line) fibroin dissolution are reported. Each I-V curve, is labeled with the corresponding gate bias voltage.²²⁶ b) Schematics of an electrode array on a free standing fibroin is reported on the right. On the left electrodes applied on cat brain are shown. In the middle, the recorded brain activity is reported. The image shows that several electrodes can acquire a good signal (green colored).²²⁸ c) schematics of wireless bacteria sensor functionalized with a graphene layer is reported.²²⁹

SF films have been used as substrates in bio-sensing devices; for instance, a wireless bacteria sensor has been developed using SF as a substrate.²²⁹ A thin graphene layer has been deposited on a SF film and functionalized with a biorecognition peptide. The freestanding device, wrapped on a tooth, could reach a limit of detection (LOD) of one single bacterium. This is the first example of a noninvasive sensor based on SF.

Recently, a new extraction process has been reported.²³⁰ This process consists in dissolving degummed fibroin in formic acid in the presence of a given amount of Ca^{2+} ions. In this way, it is possible to increase flexibility and stretchability upon tuning the amount of Ca^{2+} ions. Calcium ions, in fact, on the one hand bind to the silk protein via chelation and charge interactions and, on the other hand, can capture water molecules from the atmosphere through coordination.²³¹ Highly flexible electrodes have been developed and applied directly on the skin^{230,8}, so that electromyogram (EMG) measurements have been carried out, as reported in Fig. 3.8

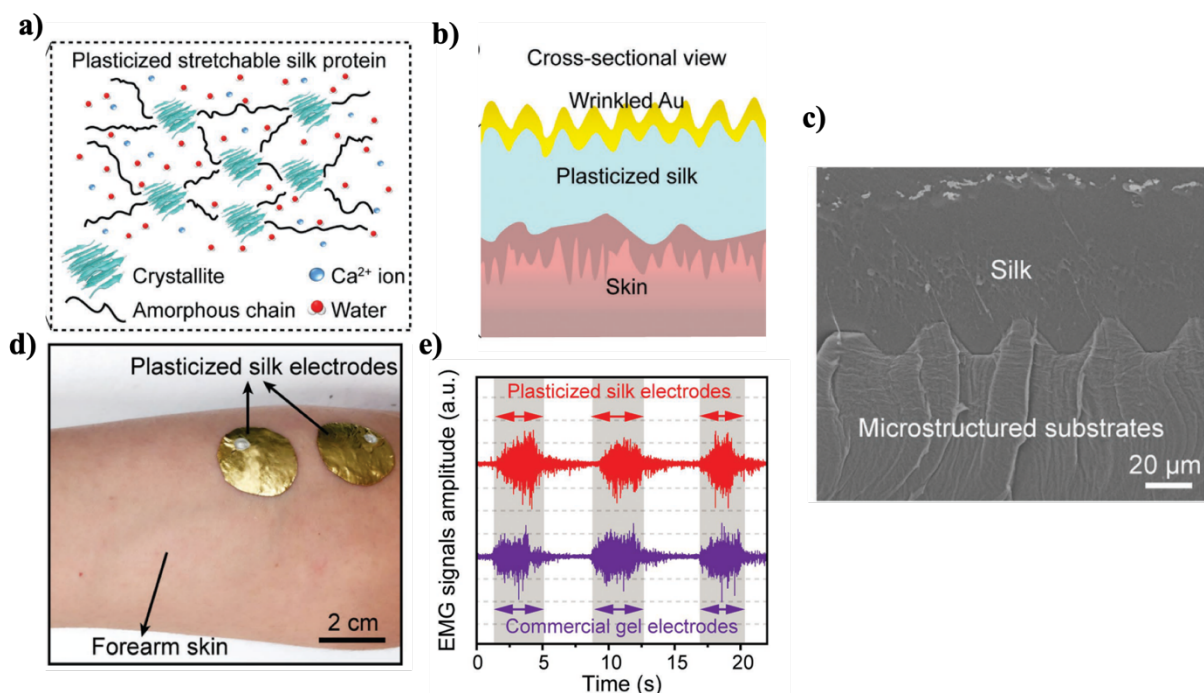


Figure 3.8: a) sketch of the internal structure of plasticized silk; and in b) schematics of the cross section of the electrode applied on skin. The electrode is composed by a wrinkled Au thermal evaporated on a free-standing plasticized fibroin. c) SEM image of the device on a microstructured substrate demonstrate the good conformability of fibroin. The electrode is applied to the skin; d) and e) EMG measurements are collected and compared with commercial gel electrodes.⁸

3.2.2 Silk Fibroin as a dielectric material for electronics applications

Silk Fibroin is a well-suited dielectric material for OTFT thanks to its stable and well-defined dielectric properties, combined with its easy processability. Accordingly, a silk fibroin film has been used as the gate dielectric in a pentacene-based OFET.²³² A high charge carrier mobility (i.e. the parameter quantifying the device attitude at implementing an effective field effect and a fast transistor switching) of $23,2 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, attributed to a better crystallization of pentacene grown on SF, has been achieved to be compared to $0,22 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ that is the mobility of a similar device fabricated on a conventional SiO_2 gate dielectric.²³³ The observed enhanced performance of such OFET is not only attributed to the better crystallization of pentacene on fibroin, but also on the role that fibroin plays in increasing the semiconductor mobility.²³⁴

This is possible because SF structure, composed by an ordered series of β -sheets crystallites in the Silk II phase, reduces the formation of defects and traps at the dielectric/semiconductor interface.

A high mobility of $0,21 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ is also achieved with devices based on Poly(3-hexylthiophene) (P3HT) at operation voltage of 3 V fabricated on SF thin films.²³⁵

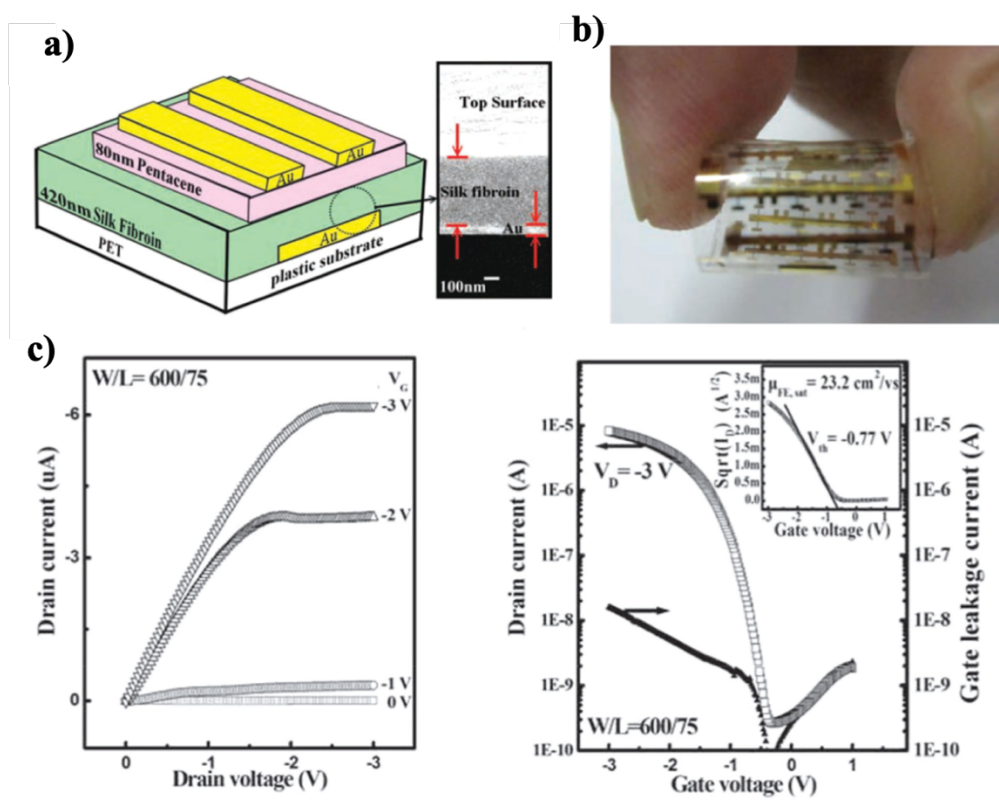


Figure 3.9: Schematic a) of the pentacene OFET with silk fibroin as gate dielectric layer. On the right a FEFSEM cross section of the device. The film is flexible b) and rollable. c) the electrical characterization is reported. On the left the output curve, on the right the transfer curve and the gate leakage current. The inset shows the $I_D^{1/2}$ vs V_g plot.²³²

The mobility enhancement can be unambiguously attributed to the use of SF dielectric layer, since also OFETs based on n-type semiconductors have shown an enhanced performance with respect to the state of the art devices based on SiO_2 . For example, an n-type OFET based on fullerene (C_{60}) as active layer and SF as gate dielectric showed a mobility of $0,014 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$,²³⁶ which can be attributed to the improved crystallinity of the fullerene by interactions with SF.

The functionalization of silk fibroin with enzymes, nanocomposites or other sensitive molecules, make possible to develop silk fibroin based sensors. For example, an OFET based on graphene has been successfully used as glucose sensors.²³⁷

SF films have been used both as substrates and gate dielectrics. The glucose oxidase enzyme has been immobilized on the SF gate dielectric layer and used to induce a graphene conductivity change under

catalytic response with a limit of detection (LOD) around $0.1\text{-}10 \times 10^{-3}$ M. Silk fibroin substrates give a good flexibility and stretchability to electronics devices, and hence fabricate electronic devices on curved surfaces, for example in food analysis. Several applications in food quality and healthcare control are reported in literature.²³⁸

3.2.3 Silk Fibroin and Photonics

Silk fibroin films are ideal for optical devices, since SF shows has a higher refractive index than water, air and other substrates, high transparency in the visible range of radiation (it presents only one absorbance peak around 270 nm, which is caused by the absorption by tyrosine groups) and, importantly, it shows easy patternability. It is worth to note that, again, even optical properties are β -sheets dependent. These properties, combined with the already outlined mechanical properties, make fibroin an interesting material in photonic applications.

SF has been actively studied as a guiding material for light radiation propagations.

Perotto et al²³⁹ reported a refractive index dependence of SF extracted from different species, upon applying a radiation with different wavelengths.

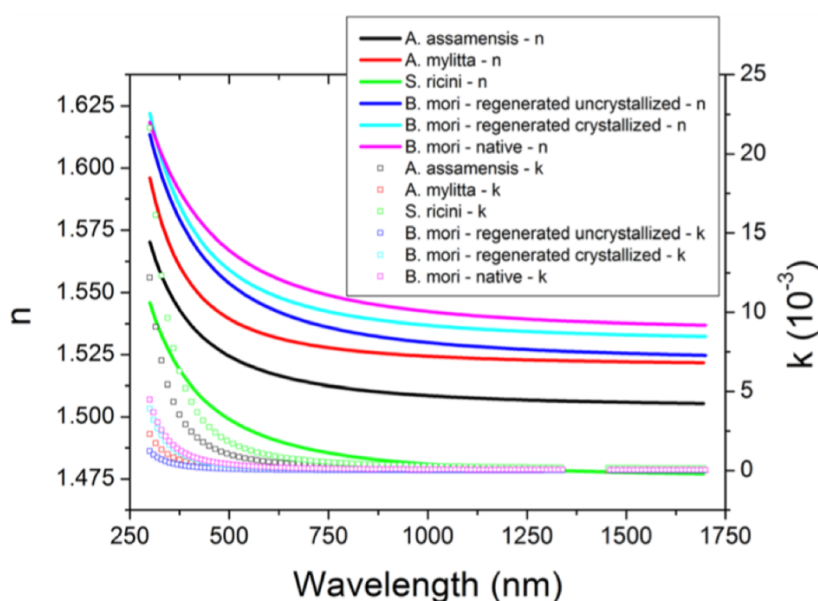


Figure 3.10: Dependence of silk refractive index from wavelength and silk typology.²³⁹

A small increase in refractive index, 1,554 to 1,555 at 500 nm, has been reported for the crystallized fibroin with respect to the un-crystallized, otherwise the absorption index k remains constant. This is caused by the increase of β -sheets fraction upon crystallization. SF films have been used to develop several optical elements and optical devices, such as optical waveguides^{223,224}, microlenses²⁴⁰,

diffraction gratings ^{241,242}, lasers ^{243,244}, light emitting transistors, inverse opals ²⁴⁵ and whispering gallery mode resonators (WGM) ²²⁵.

In 2009 Parker et al ²²³ used regenerated silk fibroin to realize printed optical waveguides on a glass substrate. The difference in refractive index between SF and glass (1,54 vs 1,52 at 633 nm) has been used to entrap and guide light along the silk waveguide, demonstrating losses below 1 dBcm⁻¹.

This value is comparable with those reported for materials commonly used for the manufacturing of waveguides, such as polymethyl methacrylate (PMMA) ($\sim 0,1$ dBcm⁻¹), silicon strip (2 ± 1 dBcm⁻¹) and others more.

Starting from the above results, an all-fibroin optical waveguide ²²⁴ where a fibroin core (n=1,54) is surrounded by fibroin hydrogel (n=1,34), is presented. The all-fibroin waveguide is coupled with a standard glass optical fiber and implanted in a bovine muscle to estimate the optical response in contact with a biological tissue. Losses around 0,5 dBcm⁻¹ are achieved. This value testifies the possibility to use fibroin optical waveguides in biomedical application, such as photodynamic therapy (PDT).

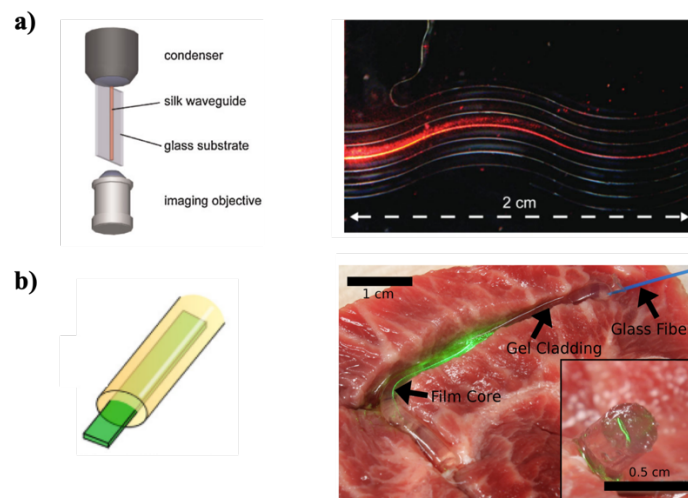


Figure 3.11: Schematic a) of silk fibroin optical waveguide on glass on the left. On the right curve silk waveguides guiding light from a laser source.²⁴⁶ On the right schematic b) of an all-fibroin waveguide. On the left silk waveguide guiding light in a bovine muscle. The light is coupled with the silk waveguide from a glass optical fiber. The inset shows the silk waveguide core.²²⁴

SF optical transparency, combined with the possibility to achieve a highly smooth material, opens the route of WGM cavity preparation. A WGM can be used in their basic configuration as tools for investigating fibroin photoelastic properties. A WGM is particular light resonator in which modes of electromagnetic radiation can be entrapped in a resonance cavity.²⁴⁷ To induce an entrapment of light

inside the cavity, a single mode optical taper fiber in contact with the cavity is used. In literature several kinds of shapes for the fabricated cavities have been reported: spheroid, toroid-shape, bottle neck and cylinder shape.

The first WGMs' cavity on fibroin²²⁵ was shaped in a toroidal form and showed a Q-Factor (quality factor, which is a measure of the damping of resonator modes and, as a consequence, depends on the amount of losses inside the resonator) around 10^5 , which is competitive with respect to the Q-Factors reported for the most studied cavities based on polymers like PDMS (10^6)⁷⁵ or PS (10^5).²⁴⁸

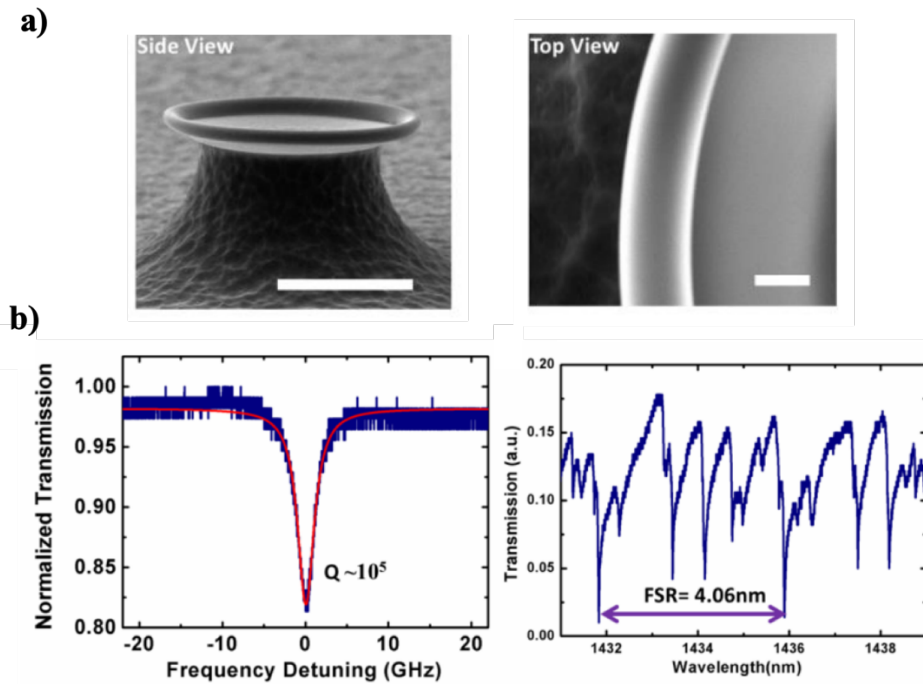


Figure 3.12: a) A SEM image of side part and the top part of toroid cavity is reported. An ultra-smooth structure is obtained. In b) the normalized transmission and related fitting for silk toroid microcavity is reported and shown a Q-Factor about 10^5 . On the left the wide spectrum range shown the free spectral range (FSR) of the microresonator.²²⁵

In this work, the silk fibroin aqueous solution was drop cast on to a PDMS smooth negative mold. After dried, silk films were peeled off and a tapered optical fiber (1,3 μm) was used to couple the micro resonators with the laser light into the fiber. A tunable diode laser 1450 nm was used to excite WGMs in the toroid resonator. Q-Factors for TE and TM modes have been determined and the system has been furthermore used as a thermal sensor, showing a thermal sensitivity for Silk II of -1,17 nm/K.

Fibroin has been shown to be an outstanding material for optical application and has been already used to make resonant microcavities to exploit their high sensitivity to a wide range of stimuli. One of the aims being to adopt fibroin to develop skin-attachable sensors for biomedical and rehabilitation applications. As already mentioned in the previous chapter, the field of wearable and easy to handle devices for all-day life monitoring is a quite relevant challenge focusing a great deal of efforts because of the very important medical and commercial implications.²⁴⁹ We therefore expanded our efforts, beyond electrochemical sensing, to optical transduction considering fibroin meshes, offering the advantages of biocompatibility, mechanical properties and suitable optical properties, as the base material for skin-attachable optical sensors. We believe that the devices developed in this thesis work represent a viable and promising strategy for widespread applications in wearable devices.

Chapter 4

Materials and Methods

The present chapter is dedicated to the description of all the experimental procedures developed for the implementation of the activity described in this thesis work.

Section 4.1 describes the fibroin extraction process by dialysis; section 4.2 introduces the method for SF (Silk Fibroin) extraction by formic acid/ CaCl_2 and fibroin films preparation. Section 4.3 discusses the procedure used to control and modify the hydrophobicity of the substrate (Kapton foils) and all the instruments used to analyze the printed features and achieve the best printing parameters for depositing PEDOT:PSS on Kapton; Section 4.4 discusses the use of these parameters for OECT fabrications and the resulting performances; Section 4.5 reports on the printing procedure developed to fabricate OECTs on fibroin.

Section 4.6 reports the set up used to monitoring ECG directly on skin with OECT fabricated on both on Kapton and fibroin.

Section 4.7 describes the procedure developed for the preparation of SF/PEDOT:PSS blend and all the techniques used to analyze the morphology, structure and the internal composition; section 4.8 presents the performance and advantages of the blend as the active channel in Organic Electrochemical Transistor. In section 4.9 shows the procedure for the fabrication of fibroin based Whispering Gallery Modes cavities on a tapered optical fiber while in Section 4.10 the set up and the optical measurement are reported and discussed.

4.1 Fibroin extraction: water procedure

Silk Fibroin (SF) batches have been prepared upon extraction from row Bombyx Mori cocoons following the original Rochwood's protocol²⁰⁴ (Fig.4.1)

According to this method, cocoons were first cut in small pieces, using a titanium scissor, and afterwards boiled in a 0.02 N sodium carbonate (Alfa Aesar) solution for 30 min in order to remove the glue-like cladding made of sericin. Degummed fibroin obtained upon separation from sericin was then washed twice under gentle stirring conditions in highly pure water for 20 min aiming at removing eventual sericin excess. Degummed fibroin was finally and left to dry under a fume hood overnight.

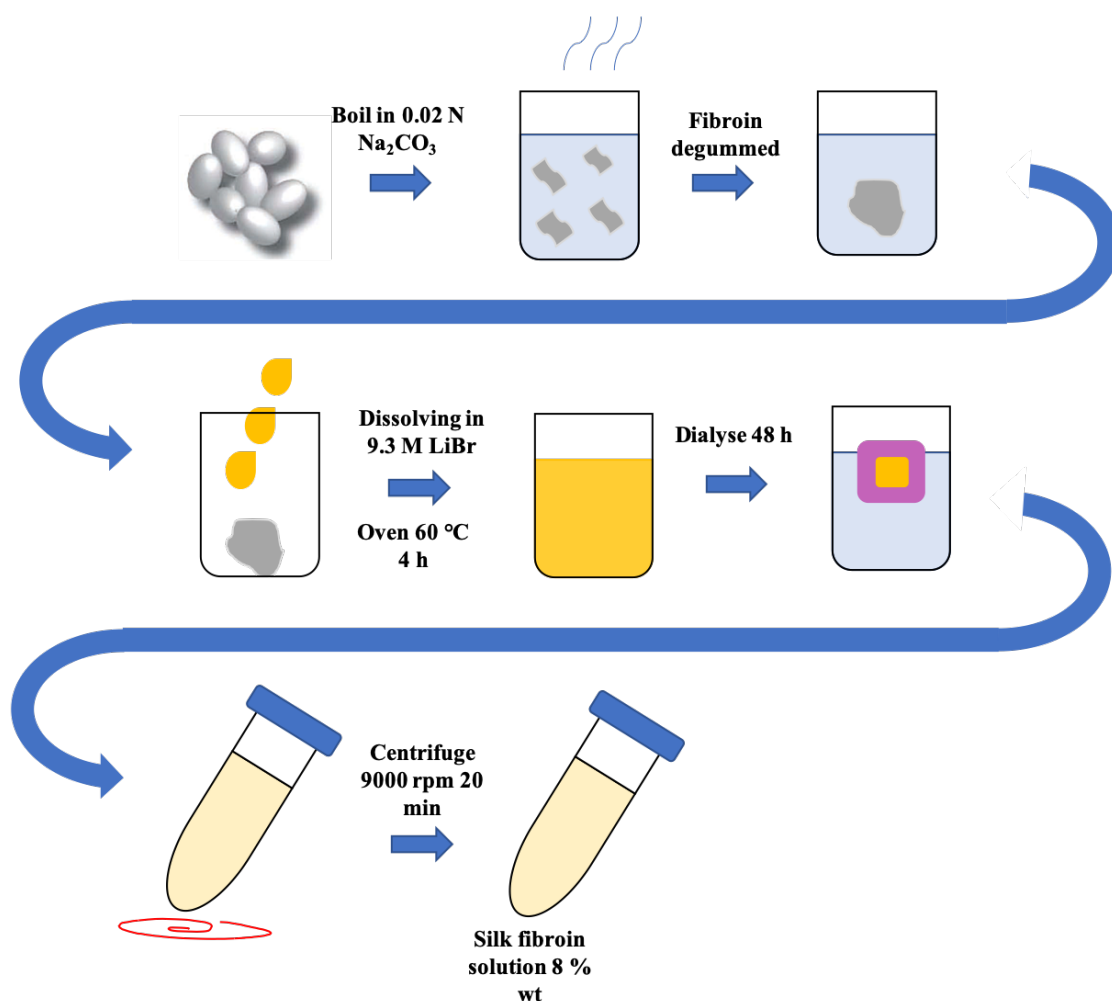


Figure 4.1: Schematic of silk fibroin extraction

Degummed fibroin was hence dissolved in 9.3 M lithium bromide (Alfa Aeser) solution for 4 h in a conventional oven at 60 °C. A slightly amber-like solution without any trace of undissolved fibroin has been achieved by the above process. After cooling down, the solution was dialyzed for 48 h in a dialyzer cassette (Slide-A-Lazer 3.5 KDa, Thermo Fischer) against ultra-pure water to remove salts. Water was changed six times during the dialysis process.

After dialysis, the SF aqueous solution was centrifuged at 9000 rpm for 20 min at 4°C to remove the unsolved portion of fibroin. This procedure was reproduced twice. A 7-8 % wt solution has been accordingly produced. The obtained solution was finally stored at 4 °C in a fridge. In this way, it is possible to prevent protein denaturation provoking the solution jellification.

To deposit the films, 4 mL of fibroin aqueous solution was cast in a 5 cm polystyrene petri dish, leaving water to evaporate under hood overnight. free-standing SF films, typically 100 µm thick. The film obtained by this procedure was mainly composed by regenerate fibroin in its Silk I form, with a large amount of random coil structures. The processes aimed at controlling the degree of crystallinity

have been already described in Chapter 3. In particular we developed a thermal treatment to induced the fibroin phase transition from Silk I to the Silk II.

The films have been annealed for 10 min at different temperatures (175 °C and 200 °C) to study the β -sheets formation and have been hence studied and compared with the pristine fibroin film by means of a wide angle XRD diffractometer (Thermo ARL X'TRA equipped with a Si (Li) solid state detector).

4.2 Silk extraction: the CaCl_2 procedure

Dried degummed fibroin, prepared as reported in the previous section, was dissolved in CaCl_2 /formic acid (FA) solution to produce a solution with the desired SF/ CaCl_2 /FA ratio. To this end we first added CaCl_2 (Alfa Aesar) to formic acid (Carlo Erba) with a 1/20 w/w ratio. Second, we added degummed fibroin to the solution at 15 %, 20% and 25% of CaCl_2 contents in weight aiming at evaluating the amount of calcium giving the optimal easy-handling films.

The solution was gently stirred to induce the complete silk dissolution. Finally, air bubbles trapped in the viscous solution have been removed using a vacuum pump.

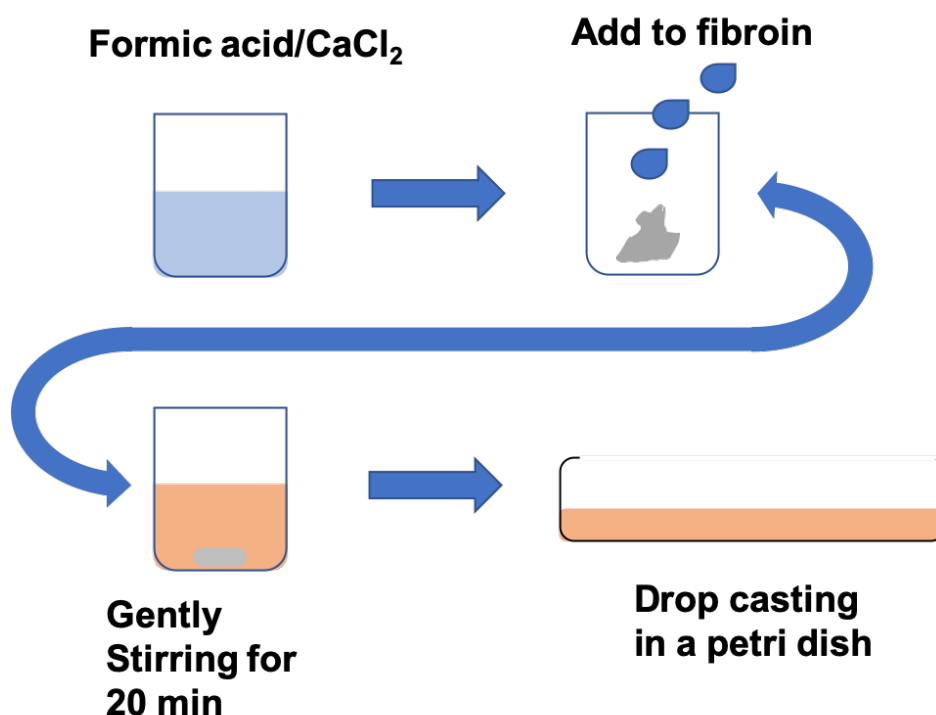


Figure 4.2: Schematic of silk fibroin extraction

4 mL of solution was cast in a petri dish and left to dry in a fume hood. The film was peeled off (100 μm thickness) and stored in a humid ambient to preserve the film properties.

4.3 PEDOT:PSS ink printing characterization

A commercial Aerosol Jet Printer (AJP 200, Optomec Inc.) equipped with both an Ultrasonic (UA) and a Pneumatic Atomizer (PA) was used for all the printing runs. An Inkjet formulation of PEDOT:PSS, suitable for ultrasonic aerosol printing, was purchased from Heraeus GmbH (Clevios P JET N V2, viscosity of 5cPs). It is an ink made of a dispersion in PSS phase of PEDOT clusters with nominal average diameter $< 20\text{ nm}$ and contains an 8% v/v of Ethylene Glycol to induce a secondary doping in the films. An amount of 2 mL of PEDOT: PSS ink was loaded in a glassy vial fitting with the specific slot inside the UA of the AJP 200 system, just before every printing run. A stabilizer water bath surrounded the vial allowing to keep the temperature of the ink constant at 20°C . The current in the ultrasonic power supply was set to a constant value of 0.5 mA in order to generate the aerosol. The Kapton foils (75 μm thickness) used as substrates. purchased from RS Electronics were cleaned with Acetone, IPA, DI-Water, followed by a plasma-oxygen (2 min, 30 W) before each printing run aimed at enhancing the hydrophilicity of the surface and, as a consequence, allowing the ink to adhere easier over the Kapton surface. Untreated substrates showed that hydrophobicity of the surface makes the ink not to adhere properly on the substrate, producing in most cases discontinuous or extremely ill-defined lines. The speed of the stage (1 mm/sec) and the distance between the tip of the nozzle and the substrate ($d = 3\text{mm}$), were kept constant during all the performed printing sessions to obtain . Preliminary tests showed that the chosen distance d was the optimal one for a better film homogeneity. Experiments with four nozzles with different diameters $\text{ND} = 100, 150, 200$ and $300\text{ }\mu\text{m}$ were carried out for printing PEDOT:PSS lines.

Printed lines have been analyzed by SEM microscopy (Carl Zeiss Auriga series Compact field emission gun scanning electron microscope), to determine both the line width (LW) and the presence of instabilities that have been quantitatively evaluated through an open-source imaging software analysis (Gwyddion). In detail, the Gwyddion analysis (roughness tool) consists of evaluating and comparing the roughness and the shape of each line parallel to the horizontal axis of a SEM image, $512 \times 512\text{ px}$ res, which defines the profiles of both the cross-sectional shape of the printed line and the substrate around the same line that is not covered by PEDOT:PSS. Both roughness and shape of the substrate/line profiles can be simultaneously obtained. A statistical analysis has been carried out averaging individual profiles of 128 different horizontal lines composing the ventral region of the analyzed SEM image.

A profilometer (Tencor P10) has been used to evaluate line thicknesses.

The extracted line parameters were plotted as a function of the Focusig Ratio (FR), which is the main figure of merit for characterizing line quality features, defined as the ratio between ShG and CG (see chapter 2). OS is another important factor qualifying the printing process. Fine control of OS is a critical step in the AJP process because it can cause overlapping of electrical paths, leading to short-circuits or to a not-well defined edge in device geometry.

4.4 Printing on Kapton, OECT fabrication and electrical characterization

OECTs were fabricated by an in-line process by the aerosol jet printer AJ200 (OPTOMECH Inc.). A device planar architecture has been defined (Fig.4.3) consisting of three layers identifying : the silver source and drain contacts (that define the active channel) and interconnects, the transistor channel and the gate electrode made of PEDOT:PSS and a protective/passivation layer made of the NEA 121 adhesive (Norland). The silver-ink (Clariant) and PEDOT:PSS formulations were printed using the ultrasonic atomizer (Nozzle diameter $ND=200\text{ }\mu\text{m}$). The silver traces were printed using a $FR=2$ by keeping the plate temperature fixed at $50\text{ }^{\circ}\text{C}$. PEDOT:PSS was printed using a FR of 2 and a plate temperature of $60\text{ }^{\circ}\text{C}$. Plate temperature is essential to facilitate the evaporation of the solvent in the generated aerosol particles at their impact with the substrate, allowing to tune and control the line quality. NEA 121 is a highly viscous adhesive, so it was printed using the pneumatic atomizer by equipping the deposition head with the largest nozzle (Nozzle diameter $ND=300\text{ }\mu\text{m}$) and setting the deposition parameters as follows: $ShG = 50\text{ sccm}$, $CG= 1200\text{ sccm}$ and $ExG= 1150\text{ sccm}$.

The ratio between the gate and channel areas of the as-fabricated OECTs was varied from 2:1 to 5:1, while the distance between the channel and gate was fixed at $150\text{ }\mu\text{m}$ for all the printed devices. For each of the gate/channel area ratio, three OECTs were printed. The aspect ratio of the designed OECT channel (width/length, W/L) was set to 1 ($W=200\text{ }\mu\text{m}$; $L=200\text{ }\mu\text{m}$). Process parameters were selected to obtain a 200 nm thicker PEDOT:PSS layer.

Electrical characterization was carried out using a National Instruments PXIe-8370 system equipped with a PXIe-4145 source measure unit (SMU). Output characteristics were acquired with V_{ds} varying from 0 V to -0.6 V and V_{gs} from 0 to 0.8 V , with a 0.05 V step and a scan time rate of 0.1 sec . Transfer characteristics were measured at fixed $V_{ds} = -0.1\text{ V}$ by sweeping V_{gs} from -0.2 V to 0.8 V , with a 0.05 V step at a scan time of 0.05 sec .

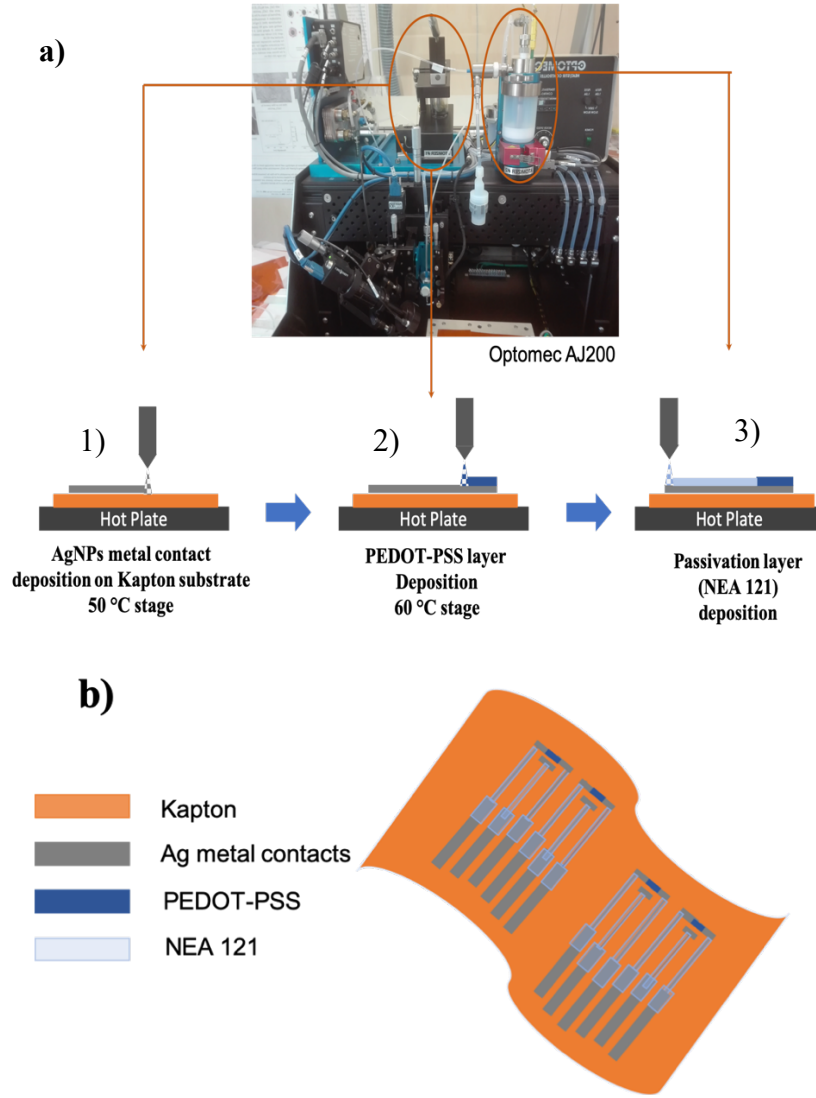


Figure 4.3: Schematic a) of printable OECT fabrication steps. In b) schematic of OECT printed on Kapton substrate

A solution of 0.1 mM of NaCl in DI water was used as gate electrolyte during output and transfer-characteristic measurements and confined by a well-made of NEA121-based printed wall ring surrounding the channel and the gate electrode. Each measurement was repeated three times and the channel was hydrated with H₂O Milli-Q after each step of measurement. to allow water-channels to form a continuous network inside the PEDOT:PSS microstructure. Transconductance g_m , which is a figure of merit measuring the OECT amplification capability, was calculated by plotting the derivative of the transfer curve ($g_m = dI_{ds}/dV_{gs}$). The transit time of holes (τ_e) in the PEDOT:PSS channel was estimated by recording the $I_{ds}(t)$ ($V_{ds} = -0.1V$) upon application of four constant gate currents, ($I_{gs} = 100 \text{ nA}$, 500 nA , $1 \text{ }\mu\text{A}$ and $5 \text{ }\mu\text{A}$).

4.5 Printing OECT on Fibroin

The fabrication by AJP of OECTs on fibroin substrates was carried out on fibroin films extracted by the CaCl_2 protocol with a 20% in weight content of CaCl_2 .

An innovative process has been developed to maintain the film hydrated during the temperature increase controlled by the AJP plate.

A PDMS (Sylgard) film was manufactured and glued with liquid PDMS on a glass petri dish. A fibroin film was positioned on the top of the PDMS film, while a wet sponge properly trimmed for surrounding the fibroin film was placed on the PDMS support. During the printing process, water was constantly supplied to the sponge, acting as a reservoir of water. In this way, the needed humidity in proximity of the fibroin film was provided to avoid the changes in mechanical properties occurring to fibroin under a drying process.

An innovative silver ink (EI-1403) purchased by Electroninks has been experimented to print the silver electrical contacts. This has been done after a careful evaluation of the optimal silver printing conditions in terms of FR values and number of stacked layers. FR were varied between 1.5 and 3.5, while different number of layers, from 1 to 10, have been tested to find the optimal silver electrical conduction.

All the essential parts of the OECT were printed under an in-line process, as shown in Fig. 4.4: definition of silver interconnects (1), deposition of active channels made of PEDOT-PSS (2) and completing the OECT fabrication by a passivation layer made of NEA 121 (3).

The silver-ink (Electroninks) and PEDOT:PSS (Clevios P Jet NV2) formulations were printed using the ultrasonic atomizer ($ND=200\text{ }\mu\text{m}$). The silver traces were printed using $FR=3$ and $80\text{ }^\circ\text{C}$ of plate temperature. PEDOT:PSS was printed using $FR=2$ at $60\text{ }^\circ\text{C}$. NEA 121 was printed with the same parameters used for the devices fabricated on Kapton. For silver traces and NEA 121 the printing speed was maintained at 3 mm/sec during the whole process.

The aspect ratio of the designed OECT channels (width/length, W/L) was 5 ($W=2500\text{ }\mu\text{m}$; $L=500\mu\text{m}$). Process parameters were chosen to obtain a 200 nm -thicker PEDOT:PSS layer.

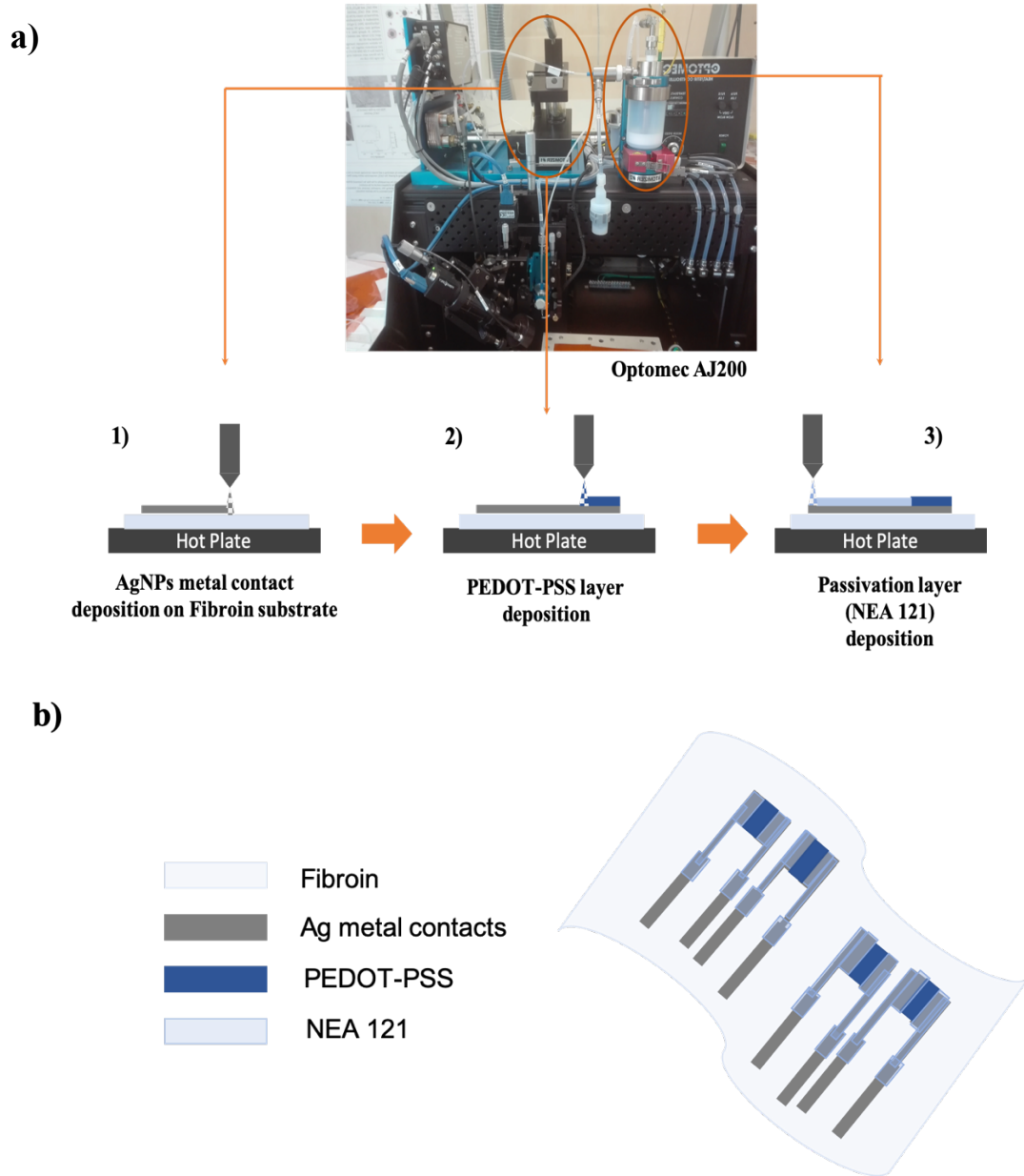


Figure 4.4: Schematic a) of printable OECT fabrication steps. In b) schematic of OECT printed on Fibroin substrate

4.6 Monitoring ECG with direct application of OECT on the skin

Commercially available systems for electrophysiology are fabricated to measure voltages while the output of an OECT is a current, hence not compatible with classic acquisition systems. For this reason, the conversion of a current output in a voltage output became necessary. To achieve this, a common source configuration where a resistor (R_{load}) is placed in series with the drain electrode, has been used. For each device (on Kapton and on Fibroin) a $R_{load} = 1k\Omega$ has been used and the whole system has

been biased by an external supply voltage applied with a DAQ system. The experimental scheme of the source common OECT electrical configuration is reported in Fig.4.5

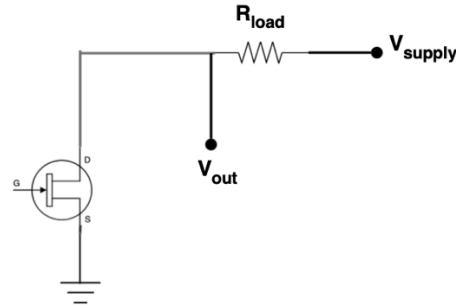


Figure 4.5: Circuit used to convert current output in voltage output

ECGs have been measured under a standard configuration typical of the clinical practice, i.e. by mounting the set up on the body using the Einthoven's triangle configuration ²⁵⁰ in which three electrodes (working, reference and noise) are placed in specific positions on the body in order to form a triangular shape. In our set up the printed OECT, acting as working electrode, has been wired on the right arm of a volunteer and two commercial Ag/AgCl (SkinAct) electrodes, acting as reference and noise subtraction electrodes, have been wired on the left arm and behind the left ear, respectively. All the three electrodes have been connected to an acquisition card (OpenBCI Cytron board) with the aim of filtering the recorded signal to remove all the noise due to both the experimental electronic network and body. The described scheme is reported in Fig.4.6.

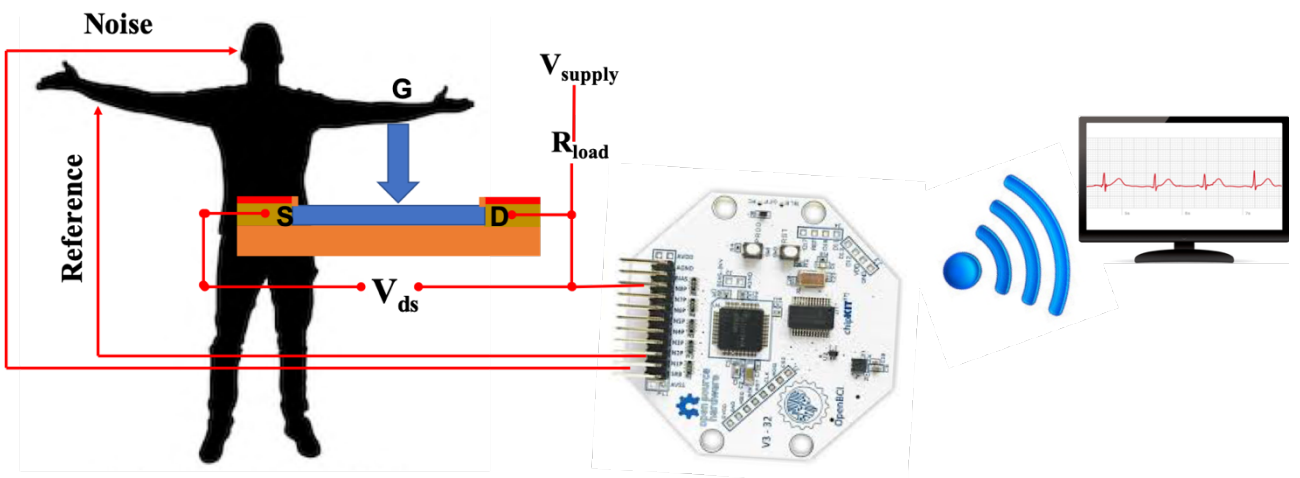


Figure 4.6: Schematic of the ECG monitoring set up

4.7 SF/PEDOT:PSS Blend Preparation

The silk fibroin solution prepared by the water based procedure described previously has been mixed with PEDOT:PSS Clevios PH 1000 (Heraeus GmbH) in a 1:1 w/w ratio. A dark blue hydrogel was obtained upon gently stirring the prepared blend for 5 min. The hydrogel was then removed and dried in vacuum for 1h.

Finally, the dry blend was further processed in a conventional oven at 50 °C for 30 min (Fig.4.7). A dark blue blend was accordingly produced. The blend morphology was studied by imaging techniques (Optical Microscopy, Leica DMS300, and Scanning Electron Microscopy, Carl-Zeiss Auriga Compact FIB-SEM). An ATR-FTIR analysis (PerkimElmer Spectrum TWO FT-IR Spectrometer equipped with a UATR accessory) was finally used to check the functional group present in the blend.

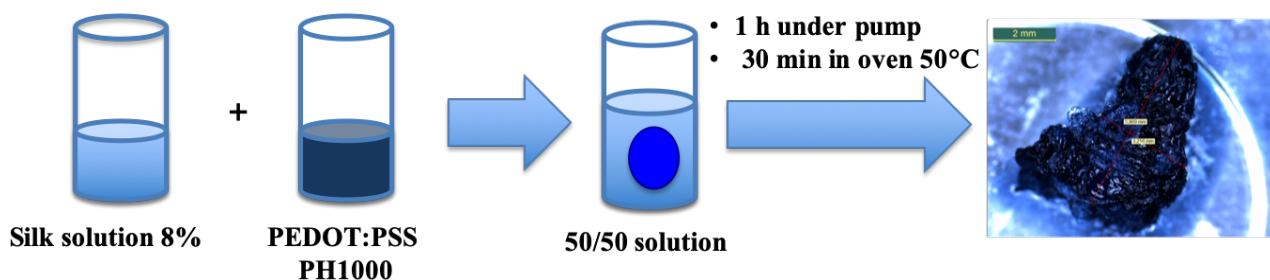


Figure 4.7: Schematic SF/PEDOT:PSS blend preparation

4.8 OECT made with the SF/PEDOT:PSS Blend and electrical characterization

The OECTs were fabricated on a silicon wafer. Source and drain electrodes, made of Ti (20 nm, adhesion layer) and Au (100 nm) bilayers, were deposited using a Kurt J. Lesker PVD 75 DC Magnetron Sputtering. The Ti/Au bilayer was patterned by a lift-off technique (image reversal AZ 5214E photoresist followed by dimethyl sulfoxide, DMSO (Sigma Aldrich), bath at 60 °C) in order to produce devices with 4 mm-long channels. The SF/PEDOT:PSS blend acting as the device channel was deposited on the fabricated test pattern. A PEO/NaCl 0,1 M has been used as a solid gate electrolyte and a silver wire as the gate electrode as shown in Fig. 4.8

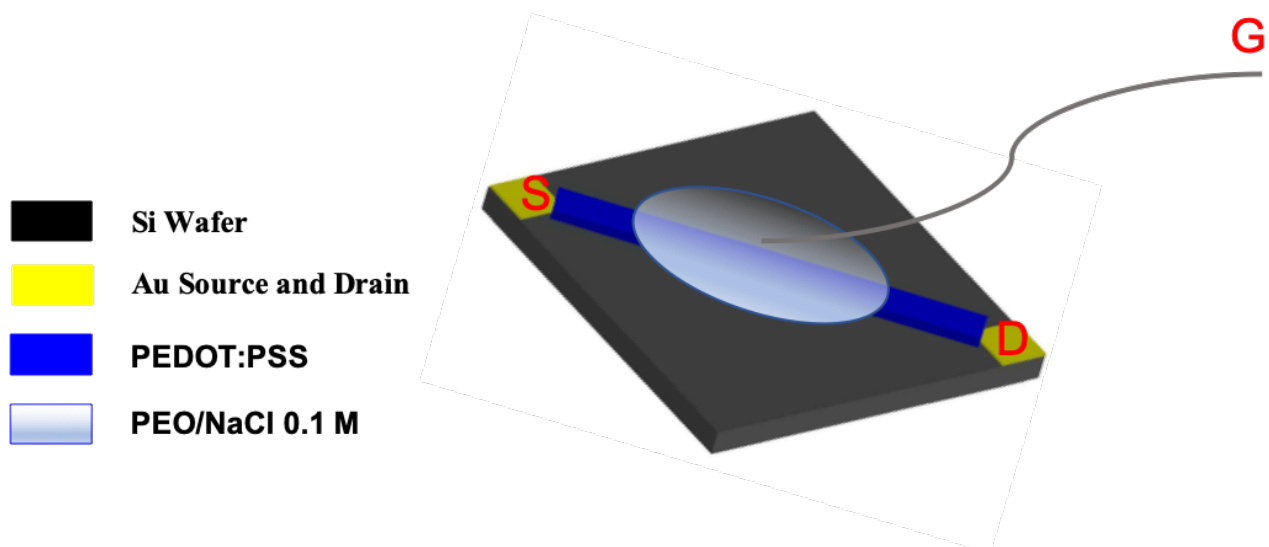


Figure 4.8: Schematic of the OEET made with the SF/PEDOT:PSS blend OEET

Electrical characterization was performed using a National Instruments PXIe-8370 system equipped with a PXIe-4145 source measure unit (SMU), used to apply the V_{ds} and V_{gs} at the OEETs terminals and to measure simultaneously the drain and gate currents, I_{ds} and I_{gs} . Transfer characteristics were measured at fixed $V_{ds} = -0.3$ V by sweeping V_{gs} from 0 V to 0.8 V, with a 0.05V step at a scan time of 0.01sec.

4.9 Silk fibroin WGM preparation

WGM (Whispering Gallery Modes) cavities based on fibroin have been prepared on optical fiber tapers fabricated from a single mode fiber SM28 (NKT) with a Vytran GPX-3000 optical fiber fuser. The preparation of WGM cavities has been implemented as follows. A taper fiber was mounted on a home-made fork and exposed for 7 min to a laser irradiation with a dose of 20 mJ/cm². A 193 nm nanosecond pulses excimer UV-laser (Braggstar) was used to induce on the fiber surface a transition from hydrophobic to hydrophilic. A double filter mask was used to create on the fiber surface an alternating path made of hydrophobic/hydrophilic regions.

A 5 μ L drop of a fibroin solution 8% wt was drop cast on the treated fiber along all its length. A set of microcavities disposed along the fiber has been obtained in this way. SEM and optical images (40x of magnification) were acquired to analyze the quality of the obtained cavities in terms of roughness and absence of collapses in their middle region.

4.10 WGM characterization and determination of the photoelastic properties of fibroin

To excite the WGMs we used a broadband superluminescent laser diode (SLD) launched into a second taper fiber (acting as the Excitation Fiber Optic) placed perpendicularly to the first fiber (Probe Fiber Optic) in the middle of the bottle-shape fibroin cavity (see Fig. 4.9)

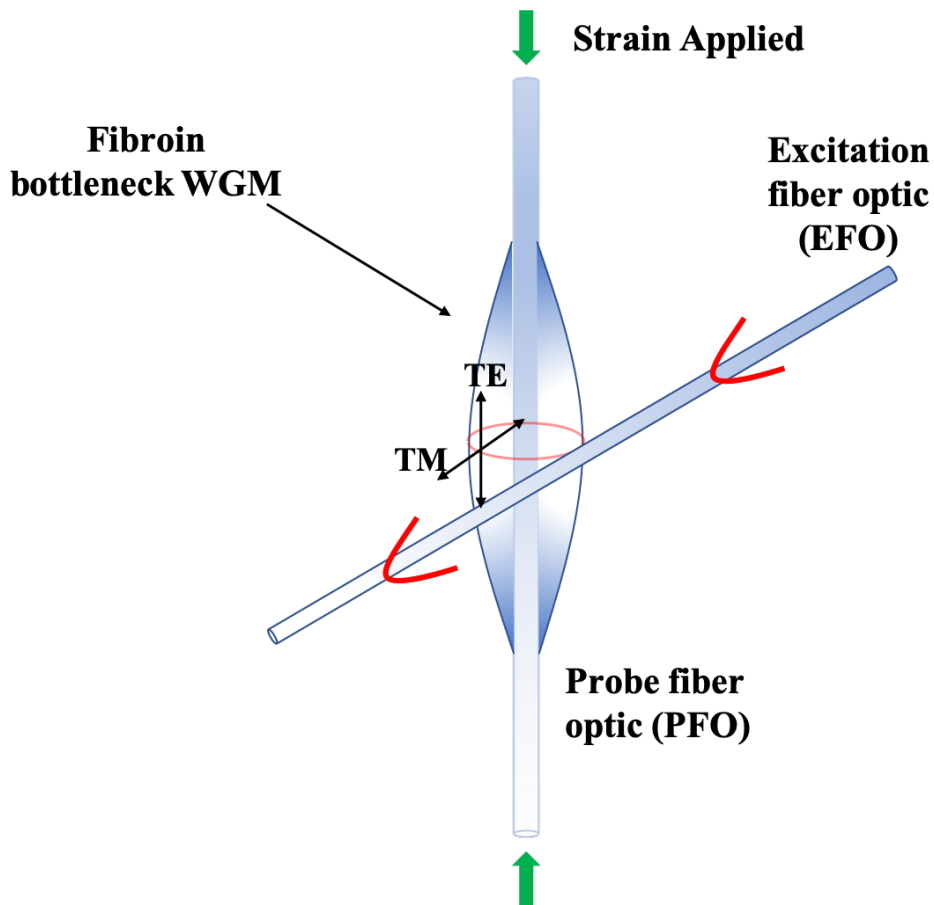


Figure 4.9: Schematic of WGM measurements set up

Transmission spectra of TE and TM modes have been separately recorded with an optical spectrum analyzer (OSA). A polarizing PANDA PM (Corning) was used to inject light inside the tapered fiber. The spectra were collected with a resolution of 0,02 nm in a wide range of wavelength, i.e. 1535-1585 nm, centered around 1560 nm (the typical telecom wavelength). The position of the Excitation Fiber optic (EFO) on the cavity surface was checked using a microscope objective.

To evaluate the optimal taper fiber diameter for the excitation and obtaining good TE and TM spectra, several diameters were tested (1.5, 2, 2.5 μm).

After spotting the optimal diameters in terms of an effective reduction of the low order modes with respect to the high order modes, a motorized micrometer screw was used to apply a strain on the WGM.

Transmission spectra of TE and TM modes were registered as a function of the applied strains with the aim of assessing the photoelastic properties of fibroin. The same experimental procedure was carried out using silk II based cavities in order to investigate the role played by the amount of β -sheet crystallizes present in the fibroin. A thermal treatment was used to induce a transition from Silk I to Silk II. In detail, the fiber equipped with the silk I cavity was mounted on a fork and placed in a home-made oven in order to perform a 10 min long annealing at 180°C.

Chapter 5

Results and Discussion

This Chapter describes the experimental results obtained along with the PhD activity and introduced in previous chapters.

Section 5.1 discussed the systematic study aimed at assessing the best printing parameters for PEDOT:PSS deposited on Kapton substrates; Section 5.2 describes the use of these parameters to fabricate an all-printed OECT and its electrical characterization. Section 5.3 discusses the interfacing of the device with human skin for ECG monitoring; Section 5.4 outline all the printing steps developed to fabricate OECTs on a silk fibroin substrate that are then used for monitoring ECG on skin; Section 5.5 is devoted to the procedure developed for the preparation of SF/PEDOT:PSS blend and all the techniques used to study morphology, structure internal composition and for its employment as an active channel in an OECT; Section 5.6 describes the thermal treatments used to induce the β -sheets structure and to tune the fibroin crystallinity index; finally, Section 5.7 illustrates and discusses the results achieved with fibroin based WGM cavities for fibroin photoelastic properties evaluation.

5.1 Printing PEDOT:PSS by Aerosol Jet technique

As already discussed in more details in Chapter 1, PEDOT:PSS is one of the best performing organic semiconductors, largely exploited in organic electronics and at the interface with biology, in particular for biological sensing and biopotential recording. Its biocompatibility and its performance in a wet environment when used as an active channel in an OECT architecture makes it easily interfaceable with biological tissues.^{251,252} Patterning of PEDOT:PSS films is usually achieved by relatively complex optical lithographic process but it can also be carried out by printing techniques such as inkjet, screen printing and aerosol jet printing (AJP). Regarding the AJP technique, the quality of the printed line depends on a number of parameters such as the wettability of the underneath substrate, deposition process parameters and ink composition, the amount of the unavoidable OS (Over Spray) effect, dust and defects on the substrates.¹⁴³

To find the best recipe for printing a material is a complex a time-consuming task, but necessary to predict the line dimensions in order to achieve structures with the desired shape, dimension and the performance.

Figure 5.1 shows a SEM micrograph of a typical PEDOT:PSS AJP line printed on a Kapton substrate (Carrier Gas flow rate (CG)=14, Focusing Ratio (FR)=4, nozzle 100 μm). The effect of overspray (OS) is clearly evident at the edges of the line as an undefined narrow profile (an example is the blue circled region, in Fig.5.1a). Similar effects are found independently from the nature of the inks used, being them based on metallic nanoparticles¹⁵² rather than on insulating, adhesive formulations.¹⁵⁸ For PEDOT:PSS printed on Kapton, the edge of the line is further corrupted by pinholes (some of them are pointed out by red arrows in Fig.5.1a), i.e. discontinuities and defects along the line edges. Pinholes are associated to surface contamination (quality of the substrate surface), or to specific solvent/substrate interactions (aqueous PEDOT:PSS suspension interacting with a hydrophobic substrate).²⁵³

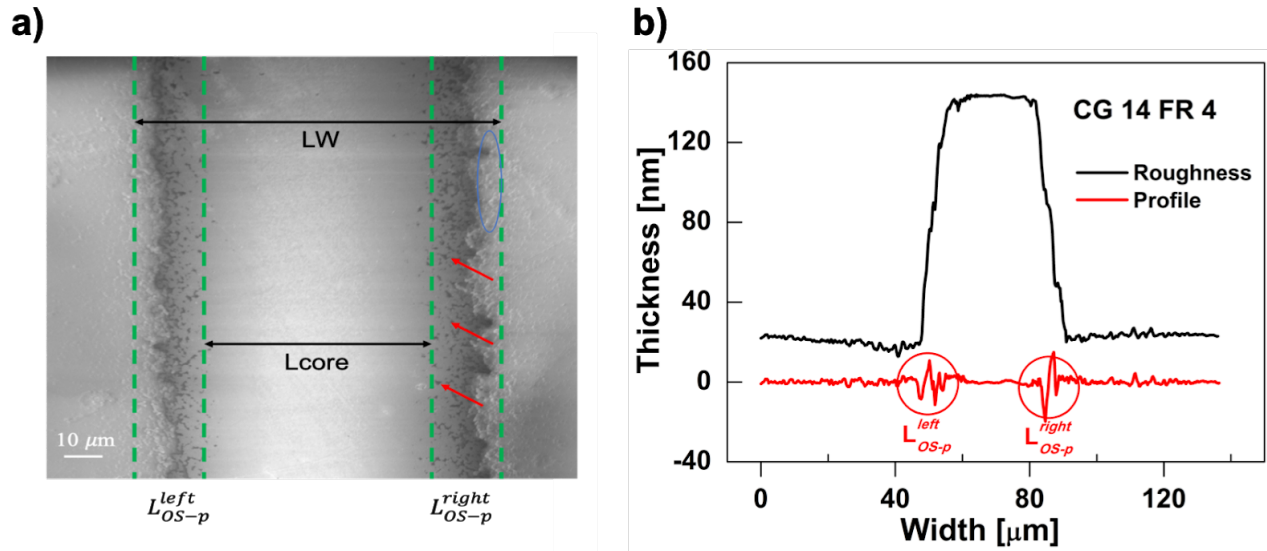


Figure 5.1: (a) SEM micrograph of a PEDOT:PSS printed line (nozzle of 100 μm , CG=14 sccm and FR=4). At the right edge, the standard OS effect (a region of which is being blue circled to guide the eye) and emerging defects (pinholes, red arrows) are highlighted (red arrows indicate a few of them); (b) thickness vs. line width profile, determined by SEM (see text) and related roughness profile. A marked change over the widths delimited between the green dotted vertical lines in figure a) is evident and are denoted as L_{OS-p}^{left} and L_{OS-p}^{right} .²⁵⁴

Figure 5.1a shows a typical printed line by a single-pass layer. As far as the pinholes is concerned, they can be removed via multi-layered printing of the same material with the drawback of drawing lines with larger thickness (additive effect) or, in some cases, increasing the stage speed.

Figure 5.1b shows the line profile of the SEM micrographs extracted by means of the Gwyddion Image analysis software, as described in Section 4.3. A pudding-like shape is charactering the PEDOT:PSS lines, which is a characteristic fingerprint of PEDOT:PSS printed on Kapton. The observed profile edge instability, i.e. percentage of OS and pinholes (indicated as OS%) with respect to the whole line width ($LW = L_{core} + L_{OS} + \text{Pinholes}$, indicated by the black line in Figure 5.1a), can be defined as:

$$OS \% = \frac{L_{OS-p}}{LW} \times 100 \quad \text{Eq. 5.1}$$

where $L_{OS-p} = L_{OS-p}^{right} + L_{OS-p}^{left}$ is the total contribution to the width of the ill-defined parts.

A criterion to assess the quality of the PEDOT:PSS lines starts from the analysis of the line width at different fixed FR and as a function of CG, repeated for the four nozzles used in this study. Figure 5.2 shows the effect of carrier gas flow on line width, where the horizontal dotted lines represent the nominal nozzle size (ND, nozzle diameter).

Data acquisition was carried out by keeping a constant CG and varying FR upon changing the Sheath Gas flow rate (ShG). LW is expected to decrease for increasing FRs, as described by the continuity equation:¹⁴³

$$\frac{LW}{ND} = \sqrt{1 - \sqrt{\frac{FR}{1+FR}}} \quad \text{Eq. 5.2}$$

Eq. 5.2 predicts, with a good agreement, the trends of LW under laminar flow conditions, especially in the case of lower CGs and for inks based on low-volatile solvents¹⁴³, therefore it is generally used for modelling different materials and substrates. The results in Figure 5.2 are well described by Eq.5.2

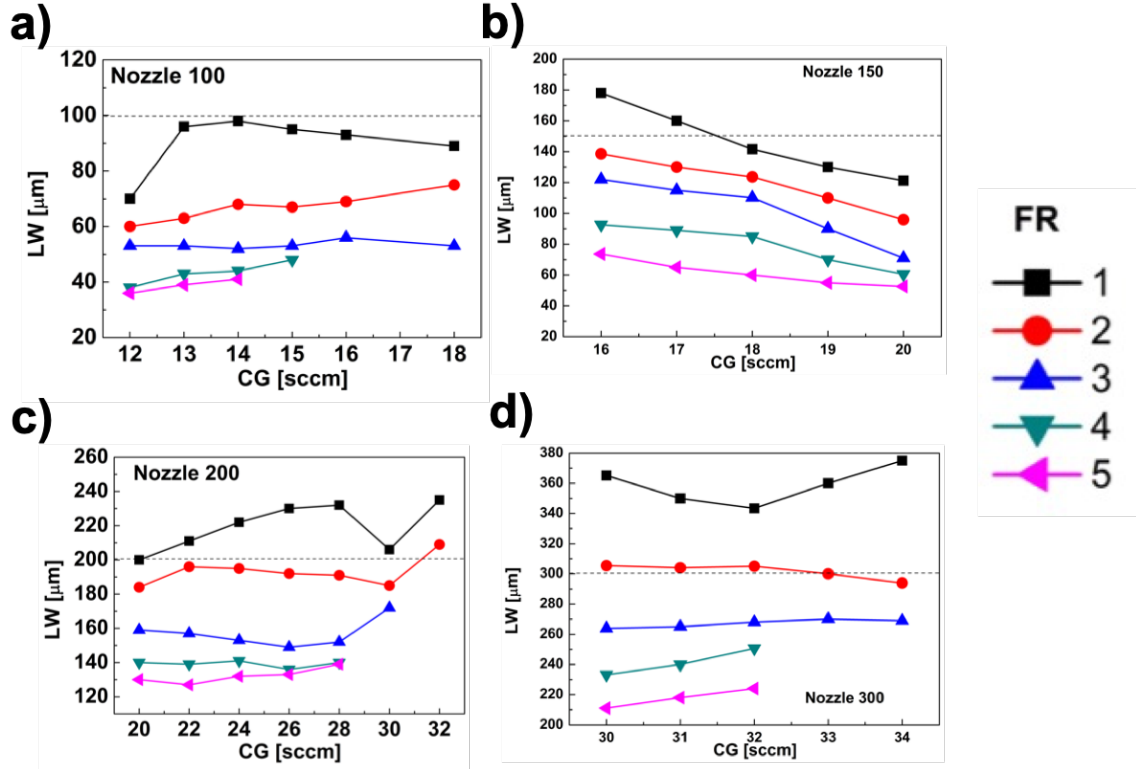


Figure 5.2: Line Width (LW) vs. CG at fixed FR ranging from 1 to 5 (color coded in the figures from black to violet). The panel shows the results for the four nozzles used with diameter size (A) 100 μm , (B) 150 μm , (C) 200 μm and (D) 300 μm respectively. The dotted horizontal line represents the nominal nozzle diameter (ND). The plots highlight the FR for which $LW < ND$.²⁵⁴

The major trends and observation coming out from our data:

- LW decreases upon increasing the FR, confirming the effects of the confinement of the sheath gas;
- the focusing effect ($LW < ND$) is observed for $FR \geq 1$ at lower ND, while there are some deviations at $FR=1$ for higher NDs (i.e. 200 and 300 μm). Such deviations are a fingerprint of the increase of $OS\%$ due to the arising of turbulences for $FR=1$ at the nozzle exit: (Fig.5.3). The aerosol stream can interact with nozzle surface defects, even though the laminar condition is fulfilled for the AJP systems¹⁵⁸ ;
- LW it strongly depends on FR, while is not conditioned by the value of CG;
- A wide range of printable widths (from 10 to 300 μm) is achieved by using the four nozzles.

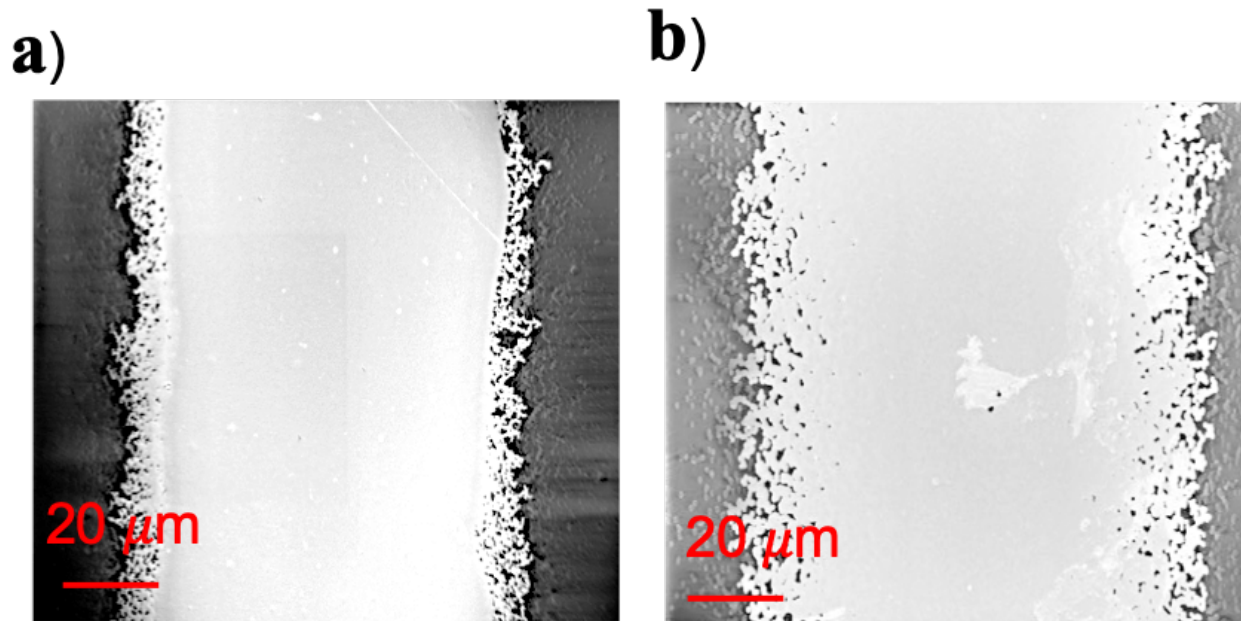


Figure 5.3: SEM image of the PEDOT:PSS lines dominated by ill-defined edges printed with $FR=1$ and nozzle diameters 200 μm a) and 300. ²⁵⁴

In Figure 5.2, have not been reported the data for FR values where $LW > ND$, since an OS-dominated printing regime takes place as it is the case for $FR > 5$. Hence, Figure 5.2 represents the PEDOT:PSS printability window, that is the region where the printed lines show minimal defects and better line edge resolution.

A typical example of a printed line dominated by OS is reported in Figure 5.4a, together with the image of two adjacent printed lines overlapping because of the excess of OS (Figure 5.4b), the image of the region between adjacent lines in case of strong OS (Figure 5.4c) and the dimensional analysis of the OS particles near the line edge, with typical drop size in the micrometric range (Figure 5.4d). The printed lines here reported are dominated by OS because the sheath gas flow pressure is chosen to be too high and a clogging of the nozzle exit is produced. ²⁵⁴

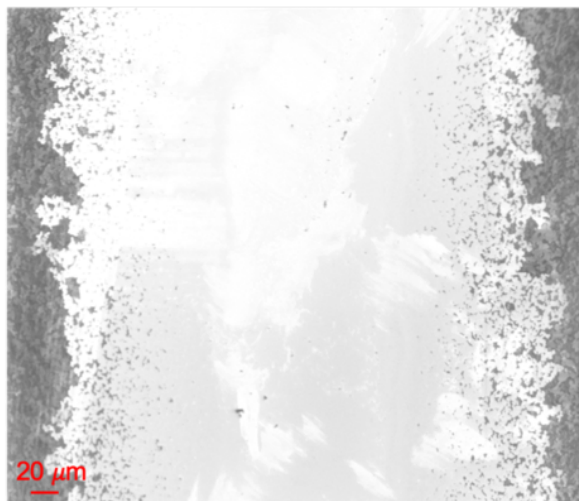
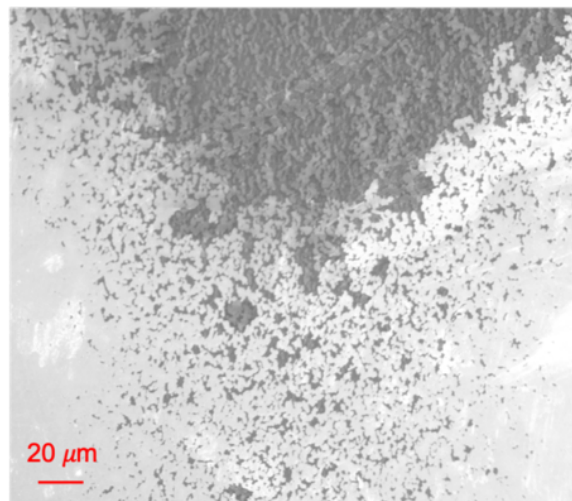
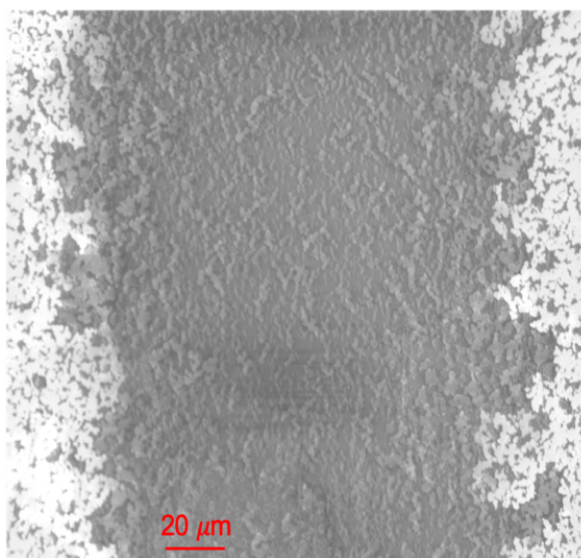
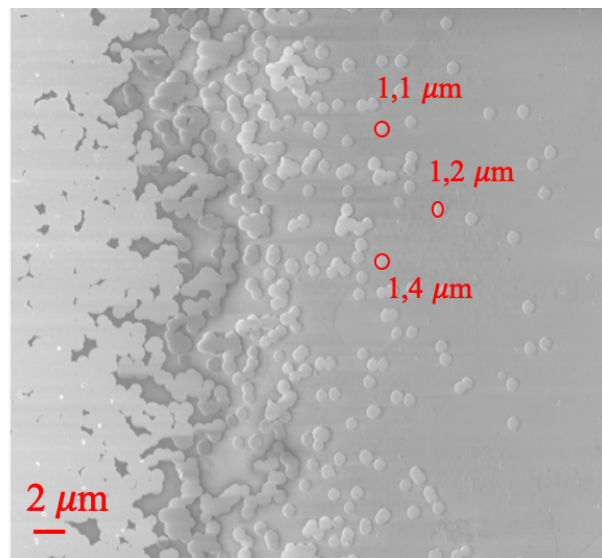
a)**b)****c)****d)**

Figure 5.4: (a) SEM micrograph of a typical printed line of PEDOT:PSS dominated by instabilities (holes in the core of line and ill-defined edges), printed with the 200μm nozzle, CG=32sccm and FR=5; (b) SEM micrograph of the region where an overlapping between adjacent lines is caused by the OS; (c) OS particles in the area between two lines and (d) dimensional analysis of the OS particles.²⁵⁴

These SEM images confirm the importance of determining the optimal parameters to minimize defects and OS issues, which are expected to negatively affect the performance of devices and in particular limiting the miniaturization of printed devices.

The second step that we have made is towards the evaluation of OS effects on a PEDOT:PSS lines. OS analysis, expressed in terms of $OS\%$ (Eq. 5.1), is shown in Figure 5.5. Here, the $OS\%$ is visualized using a polar representation where the angular coordinate represents the focus ratio (FR) and the radius length represents the OS percentage with respect to the LW. We made measurements over the whole range explored, but for a better readability, Figures 5.5 reports only the CG values corresponding to the highest and lowest $OS\%$.

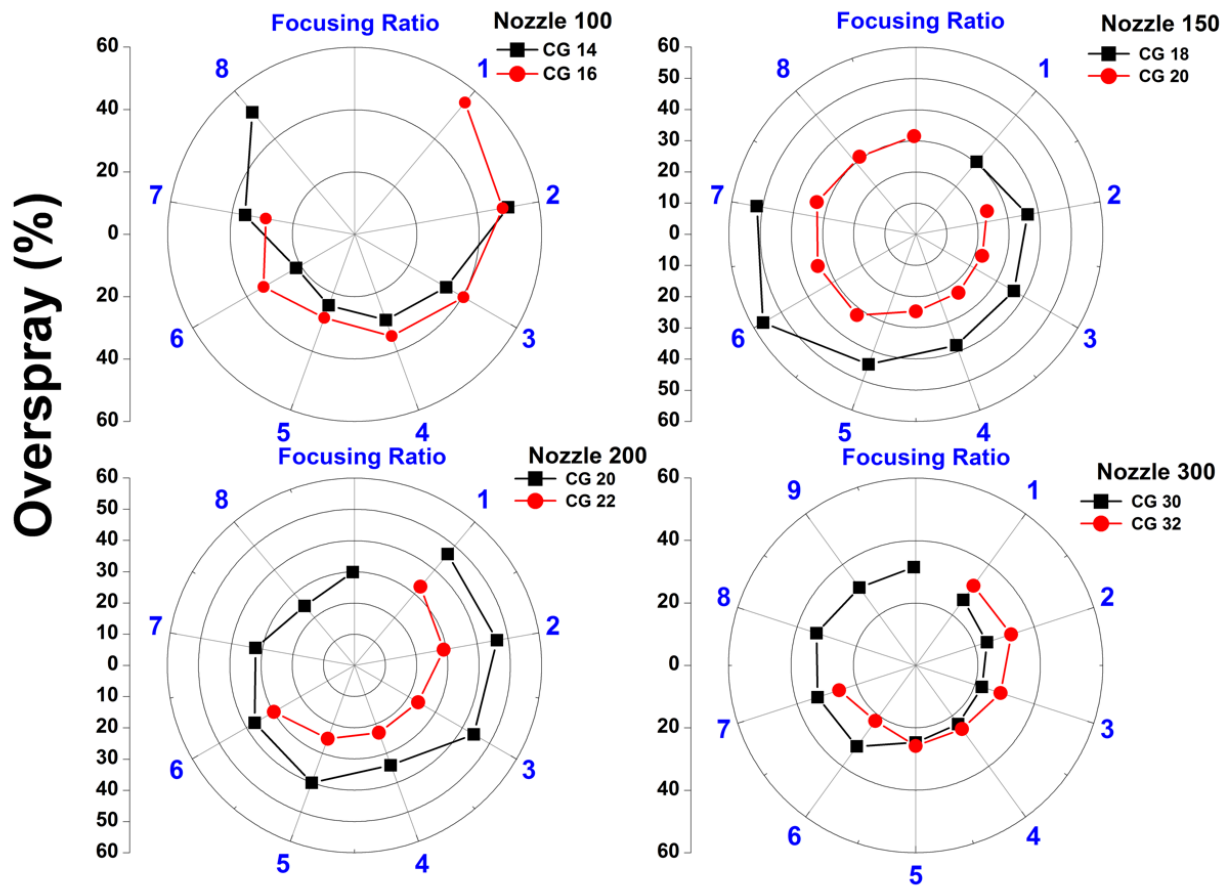


Figure 5.5: Polar representation of the $OS\%$, as a function of FR for all the nozzles used ²⁵⁴

Under this representation, the points are closer to the center of the circle, the lower is the percentage of OS with respect to LW. The overall experimental results show that the effects of OS at line edges can be reduced by increasing the nozzle diameter, the drawback being that the minimum linewidth correspondingly increase. The smallest OS percentage is achieved using the 300 μm size nozzle,

where the OS% has been found to be around 20% for each FR but the minimum linewidth become about 7 times larger than for the 100 μm . The maximum and minimum line widths as well as the OS%, are reported in Table 5.1 together with the relative CG and FR conditions.

Nozzle (μm)	Min. LW (μm) (CG,FR)	Max. LW (μm) (CG,FR)	Min. OS (μm) (%,FR)	Max OS (μm) (%,FR)
100	36 (14, 3)	98 (12, 5)	(24.2, 5)	(50, 1)
150	52.6 (16, 2)	141.6 (16, 2)	(22.3, 3)	(44.4, 5)
200	127 (22, 5)	196 (22, 2)	(22.9, 4)	(46.5, 1)
300	211 (30, 5)	294 (34, 2)	(22.3, 3)	(32.1,2)

Table 5.1: Max and min values for LWs and OS % falling inside the printability window, for each of the analyzed nozzles ²⁵⁴

The showed trends can be rationalized by means of some basic considerations coming from fluid dynamics principles. The overspray formation can be ascribed essentially to the rupture of a purely laminar flow at the nozzle exit. In fluid dynamics such conditions are modelled through the definition of the Reynolds number (Re) that parametrizes the regime in a fluid flow. In the ideal case, a laminar flow of the aerosol is always expected during its path from the atomizer to the deposition head. ¹⁵⁸ However, in the real case, the drop sizes range is expected to be wide due to the turbulence of the non-ideal AJP system. Smaller particles with insufficient inertia are not efficiently confined by the ShG, and hence they tend to deviate from the ideal nozzle axis, generating the OS issues. One of the parameters that influence Re is nozzle diameter. In particular, larger nozzles reduce turbulences and therefore limit the OS effect. At the same time, the overspray effects also depend on the drying of external particles composing the aerosol stream, taking place at the nozzle exit due to the drying effect induced by the sheath gas flow. To overcome this issue, the use of low volatile co-solvents are needed (such as Ethylene Glycol) to reduce the drying process. ²⁵⁵

The third step in our study concerns the evaluation of the dependence of the line thickness as a function of the FR.

We evaluated the thickness of the printed lines considering all the FRs in and out the printability windows. Figure 5.6 shows the width and thickness of the printed lines as a function of FR for the best CG in terms of stability over the wide FR range for the four nozzles. As in the case of silver inks, also the line thickness of PEDOT:PSS increases while width decreases. This result confirms the idea

of the sheath gas focusing. According to this phenomenon, the amount of aerosol per unit time at the nozzle exit fulfills the continuity equation.¹⁵² Hence, for a given CG, the increase of ShG results in the enhancement of the gas stream confinement effect; for this reason, the LW decreases and the thickness increases. All the LW curves depending on FR show a decreasing trend, up to a FR where a minimum is achieved. Above this FR threshold value, the trend is inverted and this occurs for all the nozzles used. The inversion of the trend is mainly due to the increase of the OS% that broadens the overall line width, confirming models proposed in literature for other materials.¹⁵⁸

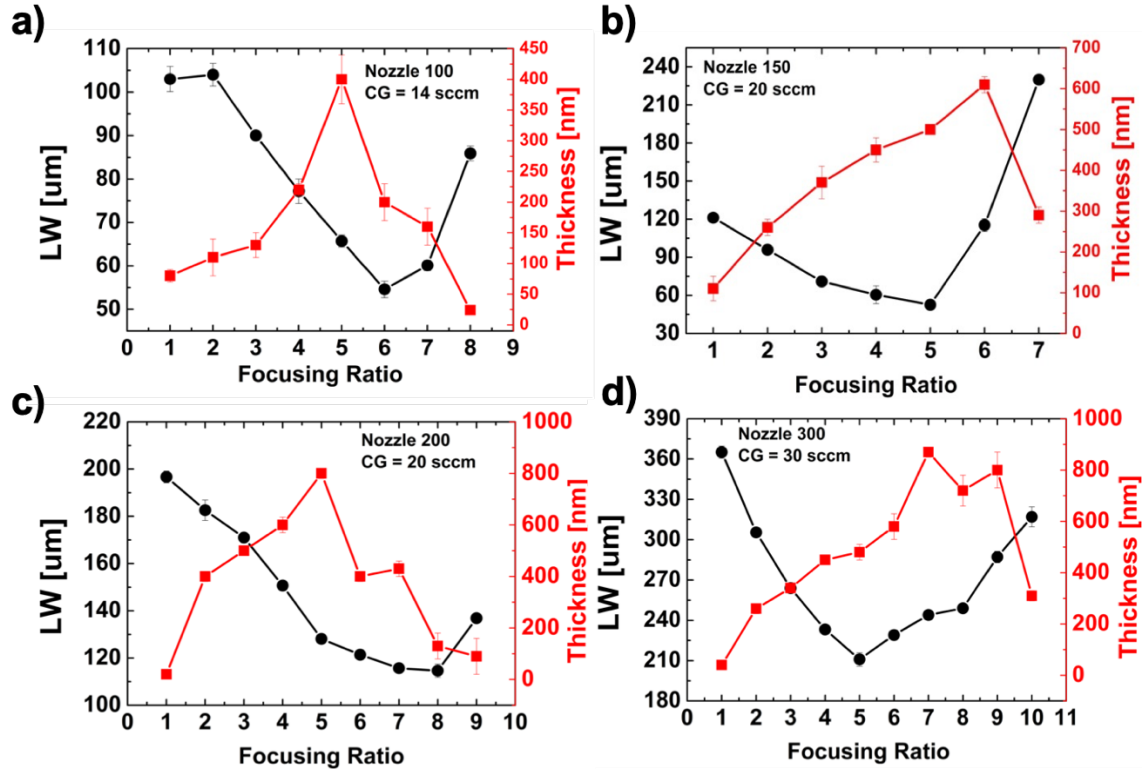


Figure 5.6: Thickness vs. Focus Ratio measured at the best process parameters achieved for each nozzle size and gas flow rate (size, CG). The trends for all the significant couples (nozzle size, CG = (100, 14), (150, 20), (200, 20) and (300, 30) are reported in the panels (a), (b), (c), (d), respectively.²⁵⁴

Moreover, from the acquired data reported in Figure 5.6, it is possible to assess the printing window for the PEDOT:PSS on Kapton, depending from selected parameters. In particular, considering the LW vs FR, the largest and most stable printing window is achieved for ND = 200 μm at CG = 20 sccm (see Figure 5.6c). As regards the thickness as a function of FR, similar thickness values are obtained in the case of ND=150 μm at CG=20 sccm (Figure 5.6b) and ND=300 μm at CG=30 sccm (Figure 5.6d), in the range of FR up to FR=6. This means that it is possible to print PEDOT:PSS lines with different widths and same thickness whenever it is required.

Finally, selecting the proper nozzle diameters and printer parameters, it is possible to select the desired thickness from a wide range: from 10 nm to 900 nm. The first value (10 nm) corresponds to a nominal monolayer of PEDOT grains in a PEDOT:PSS suspension.⁷ These results are well suited and interesting for several applications including those based on supercapacitors,^{256,257} and organic electrochemical transistors with enhanced amplification capability.⁶⁶

5.2 All-PEDOT:PSS printed OECTs

We take advantage of the ability to control printed lines features and of the knowledge of *OS* impact on line definition to print organic electrochemical transistors with properties finely tailored, on the applications they are devoted to. The results obtained in the previous paragraph, have been used to demonstrate the fabrication and characterization of a whole-organic electrochemical transistor in which both the active channel and the gate electrode are made of PEDOT:PSS.²⁵⁴ The classical manufacturing process used to fabricate OECT is based on the well-known optical lithographic technique⁷³ and only recently printing techniques such as screen printing and Ink-jet printing have been used.²⁵⁸ It is worth remembering here that, as discussed in the introduction, OECT made of PEDOT:PSS are widely used in organic bioelectronics,^{66,259–262} as highly performing biosensor,^{66,92,263–265} and recently for bio signal recording, i.e. electrocardiograms and encephalograms.^{35,93,94,97} In this research project, we developed the first set of OECTs fully printed by aerosol jet technique (henceforth called AJP OECT), a picture of which is shown in Figure 5.7. To qualify the reproducibility of the process and the degree of definition of the features, it is worth to note that the average value of the measured channel length differs by less than 2% from the nominal value (200 μm) over the whole number of the fabricated devices (Figure 5.7, left panel) that is about one hundred.

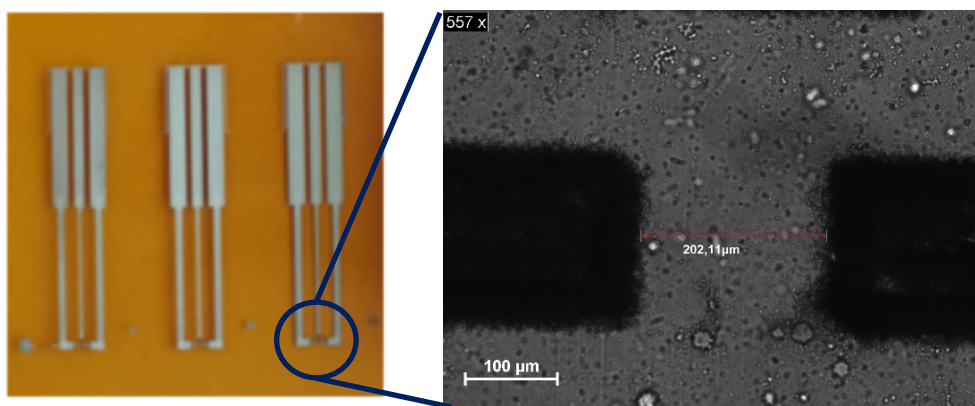


Figure 5.7: Optical images of silver electrodes defining the device channel L (202 μm)²⁵⁴

Table 5.2 reports the process parameters used for printing all OEECTs parts, where the NEA121 has been specifically used to passivate the silver pads, in order to prevent spurious effects due to the (electrochemical) interaction between the silver made electrical connections and the electrolyte used for the OEECTs characterization (NaCl):

Process parameters	Silver	PEDOT:PSS Jet N V2	NEA 121
Nozzle size (μm)	200	200	300
Sheat gas (sccm) (sccm = standard cubic centimeters)	40	60	50
Carrier flow (sccm)	20	30	1200
Exhaust flow (sccm)*	-	-	1150
Ultrasonic current (mA)	0.512	0.512	-
Platen Temperature ($^{\circ}\text{C}$)	50	60	0
Post-bake ($^{\circ}\text{C}$, time)	200, 1h	120, 1h	125, 10min

* for pneumatic atomizer only

Table 5.2: Process parameters used for the deposition of silver, PEDOT:PSS and NEA 121 inks in the fabrication of the AJP OEECTs. ²⁵⁴

A series of tests have been performed to assess the quality of printed PEDOT:PSS films through the evaluation of the material's pristine electrical current (channel current in the dry state) and charge carrier mobility (Fig. 5.8a). The channel current (I_{ds}) of pristine OEECTs has been evaluated before interfacing the polymeric channel with the electrolyte and applying a $V_{ds} = 0.1$ V between the channel contacts (source and drain).

It shows a gaussian distribution centred at 0.33 mA and a standard deviation $\sigma=0.049$ (Figure 5.8a), indicating a high functional reproducibility of the printing process.

One of the first OEECTs characterizations that has also been made is the so-called "output characteristic" consisting in measurements of the I_{ds} as a function of V_{ds} parametrized by a fixed V_{gs} values. Fig.5.8b shows the output characteristic of a typical AJP OEECT with PEDOT:PSS as gate electrode, performed by varying V_{ds} in the range from 0 V to -0.6 V with step of 0,1 V. Each curve in the figure corresponds to a different gating voltage V_{gs} spanning in the range from 0 V to 0.8 V (V_{gs} step 0.1 V).

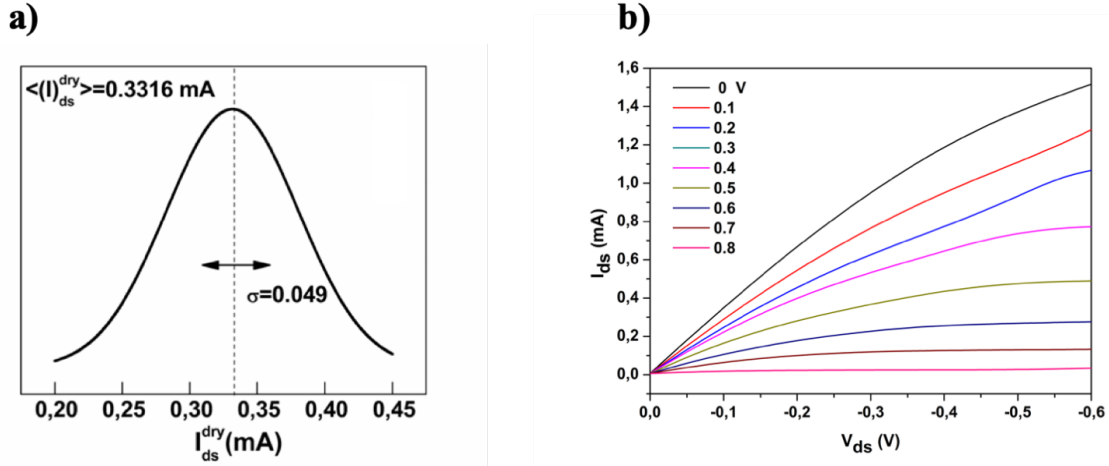


Figure 5.8: (a) Gaussian distribution of the initial channel current at $V_{ds}=0.1V$ for the manufactured OEECTs; (b) typical output curve for a 2:1 OEECT²⁵⁴

The output characteristic indicates the manufactured OEECT well perform in a low voltage regime. This is one of the most important features indicating the quite good suitability of OEECT's for application in the biological field. In this case, a good curve discrimination is achieved by varying the V_{gs} voltage after V_{ds} scan, confirming that the device works quite well even at low V_{ds} voltages. To obtain highly stable and fast responding OEECTs, it is necessary to understand which are the limiting factors and hence how to properly optimize the device architecture. In a first approximation, the time response of an OEECT depends on both the ions migration from the electrolyte to the polymeric channel and the transit time of the holes in the channel.

The holes transient time τ_e can be calculated by driving the AJP OEECT with a fixed gate current in according with the model proposed by Kodagholy:

$$\frac{dI_{ds}}{dt} = - \frac{I_{gs}}{\tau_e} \quad (\text{Eq. 5.3})$$

The holes transit time for devices fabricated and characterized in this work, (Figure 5.9) turns out to be about 12 msec (by fitting the experimental data reported in Figure 5.9 by using equation 5.3). The corresponding charge carrier mobility hence in the range of 10^{-4} $\text{cm}^2/\text{V}\cdot\text{s}$, about one order of magnitude lower than the best PEDOT:PSS OEECTs reported in literature and fabricated in a clean room and patterned using photolithography with a suspended gate electrode made of standard Ag/AgCl.^{35,74} There are no reports in literature dealing with holes mobility evaluation hasn't been reported in the literature about printed PEDOT:PSS OEECT so that our results are the first on Kapton.

We hence believe that our results are very competitive and promising if one considers some major differences in architecture and the materials used (see Table 5.3).

Substrate	Gate material	Gate position	Technique	Charge Carrier Mobility ($\text{cm}^2/\text{V}\cdot\text{s}$)	Ref.
Glass	Ag/AgCl	Vertical	Photolithography	5×10^{-2}	Khodagholy et al ²⁶⁶
Glass	Ag/AgCl	Vertical	Photolithography	10^{-3}	Bernard et al ⁷⁴
PET	PEDOT:PSS	Planar	Ink-Jet	$7,8 \times 10^{-3}$	Basiricò et al ²⁶⁷

Table 5.3: Charge carrier mobility for OECT available in literature

In particular the PEDOT:PSS formulation used for our printing systems is less viscous and less conductive than the mentioned papers; a second factor is related to the in-plane configuration that we adopted for the gate electrode to have a fully printed OECT, which is known and expected to give rise to a weaker coupling with the active channel compared to that obtained by vertical gates immersed into the electrolyte. Another factor known to play a role is the gate electrode material that in our case is made in PEDOT:PSS, to produce an all printed system with the least amount of different materials, and is well known that Ag/AgCl is better performing. Also, the substrate and the deposition technique used plays a relevant role in the hole mobility, in fact with photolithographic techniques and rigid substrate is simpler to achieve high mobility. In our case, flexible substrate, planar gate and PEDOT:PSS gate we achieved a result comparable with the $3.7 \times 10^{-3} \text{ cm}^2/\text{V}\cdot\text{s}$ measured by Basiricò et al ²⁶⁷ where the substrate was a Polyethylene Terephthalate (PET) and the gate consisted of four layer of PEDOT:PSS

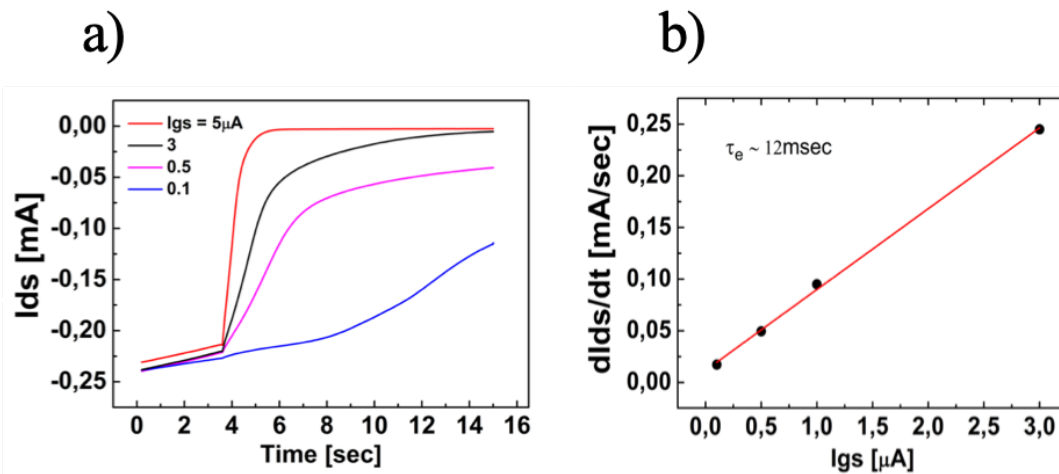


Figure 5.9: a) $I_{ds}(t)$ vs time curves recorded by current-driving the gate terminal; b) fit to equation

We have also carried out the characterization by measuring the “transfer curve”, where the I_{ds} modulation is shown as a function of V_{gs} at fixed V_{ds} . From the output curve $V_{ds} = -0.1$ V has been set as the fixed channel voltage during the transfer curve acquisition. V_{gs} has been swept from -0.2 V to 0.8 V with a step of 0.05 V.

In the transfer curve, in particular for in-plane configuration adopted, the area of the gate electrode compared to the area of the channel plays a quite relevant role. The transfer curve shown in Figure 5.10a, acquired for the OECT with 2:1 gate to channel area ratio, is a curve obtained by statistically averaging the data over a total of nine measurements (three transfers curve measured for each of the AJP OECTs having the same gate/channel area ratio). The very low magnitude of error bars (statistical average error, SAE evaluated as $\sigma / \sqrt{N}$, with $N=9$) shows that our OECT have a quite high reproducibility..

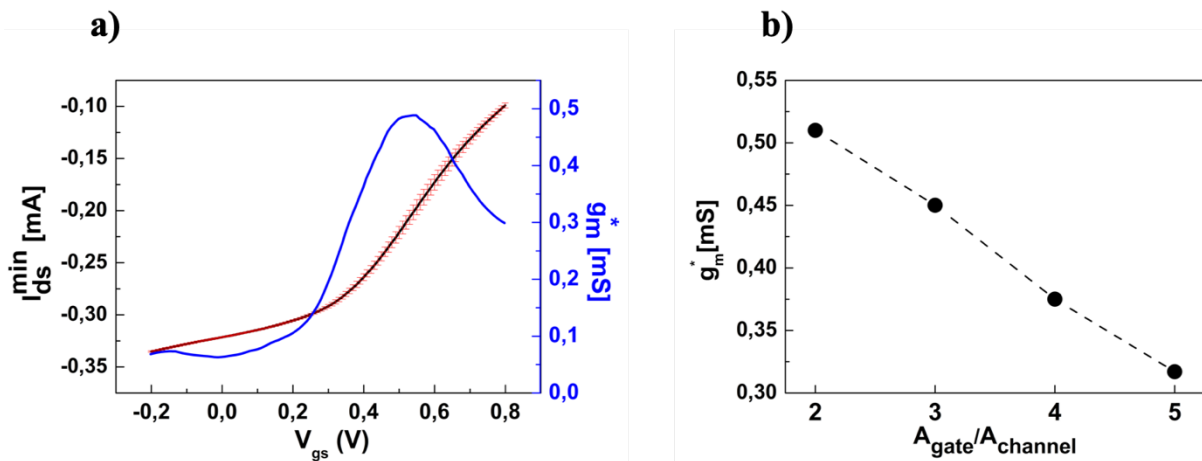


Figure 5.10: (a) transfer characteristics acquired for $V_{ds} = -0.1$ V, the right y-axis shows the corresponding calculated transconductance and (b) transconductance peak g_m^* (see text) as a function of the gate to channel area ratio ($A_{gate}/A_{channel}$).²⁵⁴

The current modulation, upon switching the gate voltage-driven cationic injection, provides ON/OFF ratios between 1 and 10, in line with the range (1 to 10) reported for planar OECTs.^{268,269,270}

From the transfer curves we assessed the main figure of merit for OECTs, describing their amplification efficiency, i.e. the device transconductance. This parameter, defined as $g_m = \Delta I_{ds} / \Delta V_{gs}$, measures the OECT’s amplification factor upon the ion to electron transduction by PEDOT:PSS.²⁷¹

We achieved a maximum device transconductance of ~ 0.5 mS at V_{gs} of 0.5 V, for the 2:1 OECT, and this is in agreement with values reported in literature (in the range of 0.2 mS for a similar architecture

made by screen printed technique).²⁶⁴ The maximum peak of g_m linearly decreases with the increase of the gate to channel area ratios (Figure 5.10b). The linear decrease suggests an additive contribution at the gate electrode that could be ascribed to the electroactive nature of the PEDOT:PSS gate electrode in presence of Cl^- ions in the electrolyte solution. During the V_{gs} operation, the anions are forced into the PEDOT:PSS bulk. This can change the microstructural volumetric (hence, additive) capacitive contributions due to electric double layers formed at the PEDOT cluster/PSS interface.²⁷²

5.3 Organic Electrochemical Transistor (OECT) for *in vivo* bio-signal monitoring

As discussed in Chapter I, Organic Electrochemical Transistor are the most used electronic devices for biosensing and for interfacing biological systems, because of their low operating voltage (< 1 V), with the unique feature of ion to electron amplifying transduction. Several applications have been reported for sensing and biosensing of a variety of biomarkers and biomolecules, *in vitro* and *in vivo*^{264,263}. A rising field is organic bio-electronic where these devices are playing a major role for bio signal monitoring.^{9,273}

Monitoring the electrical activity of live organs is a consolidated method to monitor their integrity and to understand potential diseases. In clinical applications, Bio-signal monitoring of organs and cells are fundamentals to predict diseases and possibly resolve them in time before major damages occur. Most of such signals are at low frequency (< 100 Hz) in a quite low voltage range typically from ten microvolt to millivolts.²⁷⁴ The most simple and studied bio-signal is the cardiac electrical activity, that is a very good indication of correct operation or type of malfunctioning of the hearth, that is characterized by a voltage peak to peak in the range of mV when measured on the skin.⁹

The quality of the extracted signals depends on the monitoring set up and on the material composing the electrodes. The typical commercial electrodes for electrocardiogram (ECG) monitoring are made of Au or Ag/AgCl with the need of interfacing to the skin with a conductive paste (gel type) that has the function of ion reservoir playing the role of reducing the electrode impedance at the interface, which is the principal physics parameters that determines the quality of the recording.

A low impedance is critical for ECG recording and hence there is the need to increase the electrode area. There is a growing interest in making wearable devices that could monitor in real time the heart that would require microelectrodes to be as embedded as possible. Large electrodes represent hence a serious limitation to this type of application that respond to a growing interest.

To overcome this problem and achieve, at the same time, a low impedance, several strategies have been developed such as the use of polymeric materials as electrodes (PEDOT:PSS) over flexible and possibly stretchable substrates to achieve a good conformability with skin.^{275,12}

OECT devices represent a potentially ideal solution as a prominent electrical probe for bio signal monitoring directly on skin, because of the mentioned their marked ion-to-electron transducing capability combined with the development of innovative fabrication processes such as printing technologies on several kinds of flexible and conformable substrates.

With these applications in mind, we interfaced the OECTs fabricated and studied as described in the previous paragraphs with the body for *in vivo* ECG monitoring. ECGs have been measured upon standard configuration used in clinic, mounted the set up on human body in the standard Einthoven's tringle configuration ²⁵⁰ where three electrodes (working, reference and noise) have been placed in the specific body positions to form a tringle.

In our set up the printed OECT, acting as working electrode, has been wired on the right arm and two commercial Ag/AgCl electrodes, acting as reference and noise subtraction, have been wired on the left arm and behind the left ear, as reported in Fig. 5.11

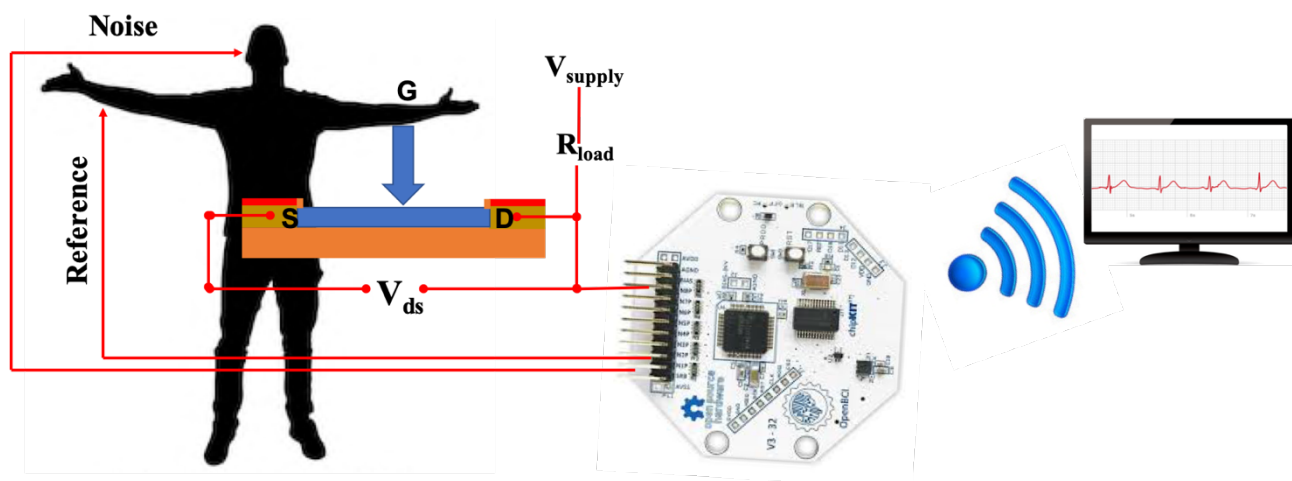


Figure 5.11: Schematics of the printed OECT connections for ECG monitoring

Monitoring bio signals, in particular ECG, through the use of an OECT presents two fundamental issues. First of all, the background noise. Noise could be either of biological origin or can be generated from the electronic devices and electronic systems involved. For these reasons, a filter system is needed cut down all the different noise signals. In this experiment an acquisition board has been used to filter the signal coming from the OECT.

Another question arises from the fact that the majority of commercially available acquisition systems for biological signals are fabricated to measure difference in voltage while the output of an OECT is current and hence is not directly compatible with classic acquisition systems. At this stage of the work

we were willing to use standard electronics to compare our results to the available standard electrodes hence we decided to convert the current output in a voltage signal by applying resistor (R_{load}) on the drain in a common source (amplifying) configuration.²⁷³

In this study, the OECT has been used under a common source configuration, by connecting it directly to the board together with the Ag/AgCl reference and noise electrodes. The electrical potential of the body drives hence the gate current and a $V_{ds} = -0,1$ V has been applied between source and drain electrodes to generate the current in the channel. This V_{ds} value corresponds to the maximum transconductance calculated for our devices at a very low gate voltage bias (i.e. the potential generated by the heart activity).

The typical ECG recorded with one of our OECT device (Fig.5.12a) well gives to the expected electrical activity of the human heart.

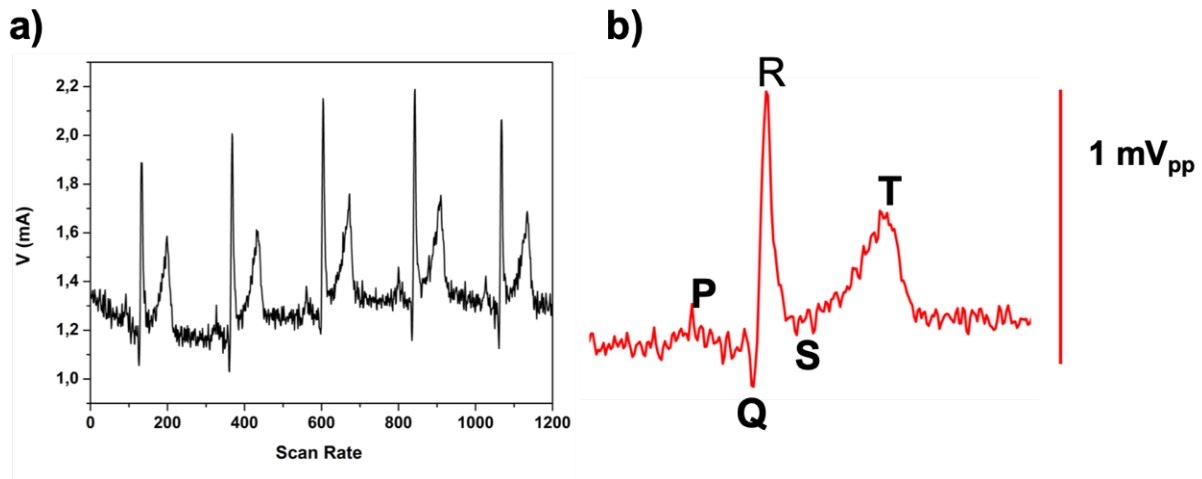


Figure 5.12: In a) the real time ECG signal recording with an all-Printed-OECT on a Kapton substrate. In b) the magnification of the ECG signal with a 1 mV_{pp} amplitude of the main peak QRS.

All the fundamental feature has been nicely recorded and shown by our OECT.

All the fundamental features of the ECG can be easily recognized in the acquired signal. The main peak QRS, that corresponds to the depolarization of the ventricles, shows a peak-to-peak voltage around 1 mV_{pp} (Fig.5.12b) that is equal to the value registered with passive electrode such as Ag/AgCl.³²

Here we demonstrate that our device presents a performance competitive with best to that of some OECTs on flexible substrate fabricated by photolithography reported in literature.²⁵²

Further experiments based on the optimization of the common source configuration aimed at showing an amplified signal, are still in progress.

5.4 Prototyping printed OECT on Fibroin: a novel perspective for bio signal monitoring

To improve the quality of the ECG signal recording *in vivo* and directly on the skin, optimized substrates in terms of flexibility, stretchability and conformability are needed.

We chose Fibroin as the best candidate on the basis of its peculiar properties.¹⁹² Different research groups have used fibroin as a substrate for several electronic devices e.g. OFET and IGFET.^{276,277}

In this work, fibroin has been used for the first time as a substrate for OECTs fabrication by the AJP technique.

Silk fibroin films are prepared using the two different extraction processes discussed in details in Chapter 4 and tested simultaneously in order to find the optimal substrate for OECTs fabrication by the printing technique. The films were formed by dehydration by thermal treatment (see paragraph 4.1) show the tendency to become rigid and brittle, and they have a very low tendency to re-adsorb water. Furthermore, they also do not present an effective self-adhesion on skin.

On the other hand, Fibroin calcium modified films remain quite well hydrated with a very nice tendency to absorb water without an easy dissolution and hence show a quite remarkable adhesion to the skin without the need of any further interfacing material. We hence developed our strategy on this type of Fibroin as the optimal substrate. Using the developed procedure described in Section 4.2 Chapter 4, a free standing stretchable, flexible and self-adhesive on skin film has been achieved. The calcium modification allows increasing flexible and stretchable character as desired upon an *ad hoc* tuning the calcium ions content (Fig.5.13). This is because calcium ions bound to the silk protein via chelation and charge interaction can capture water molecules from the atmosphere through coordination.⁴¹ Hence, calcium modified fibroin films present a self-adhesion on skin that is due to the coordination of water molecules present on the skin sweat because of transpiration.



Figure 5.13: calcium modified fibroin presents a good stretchability, flexibility and self-adhesion on skin

The main problem faced upon deposition of Ag and PEDOT:PSS for the fabrication of the OECTs on fibroin substrate is an over drying effect during the printing process requiring heating over 60°C, that causes the substrate to become too rigid and brittle. To overcome this problem, a homemade procedure has been developed, consisting of the use of a wet spongy inset surrounding the fibroin film with the aim of maintaining it hydrated during the printing process. This setup worked quite nicely.

The second problem is the over dry during the sintering process of the silver ink after printing, and for this reason an innovative silver ink purchased by Electroninks, with a sintering process at lower temperature around 100 °C for 30 min, has been selected.

Table 5.4 reports the process parameters used for printing all OECTs parts on the fibroin substrate:

Process parameters	EI-1403	PEDOT:PSS Jet N V2	NEA 121
Nozzle size [μm]	200	200	300
Sheat gas [sccm]	30	60	50
Carrier flow [sccm]	15	30	1200
Exhaust flow [sccm]*	-	-	1150
Ultrasonic current [mA]	0.4	0.512	-
Platen Temperature (°C)	80	60	0
Post-bake (°C, time)	100, 30 min	120, 1h	125, 10min

* for pneumatic atomizer only

Table 5.4: Process parameters for AJP deposition of the three inks used in the fabrication of the OECTs on fibroin substrate that is silver, PEDOT:PSS and NEA 121.

Figure 5.14 shows the real image (photo) of the all-printed OECT on fibroin with a W/L= 5

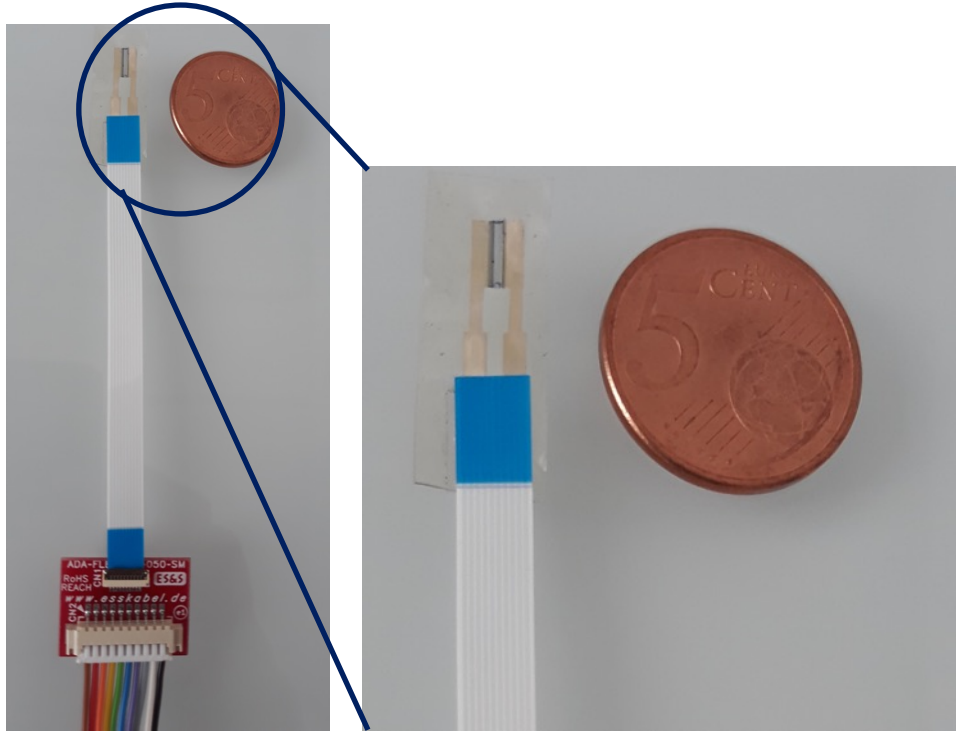


Figure 5.14: Real image of the all-printed OEET on fibroin connected with a ZIFF interconnection to the electronics. The OEET is fabricated with a PEDOT-PSS channel with a $W/L= 5$ dimension.

The printing OEET has been interfaced with human body using the same set up used for the OEET on the Kapton substrate. Thanks to the self-adhesion of the fibroin substrate to the human skin no medical tape has been used to stick the device on the body in contrast with the case of OEETs on Kapton. For the ECG signal monitoring a $V_{ds} = -0.1$ V has been supplied between source and drain electrodes. Also, in this case, the body's electrical potential acting directly as gating signal.

Fig. 5.15 shows the measured ECG monitored with the OEET on fibroin

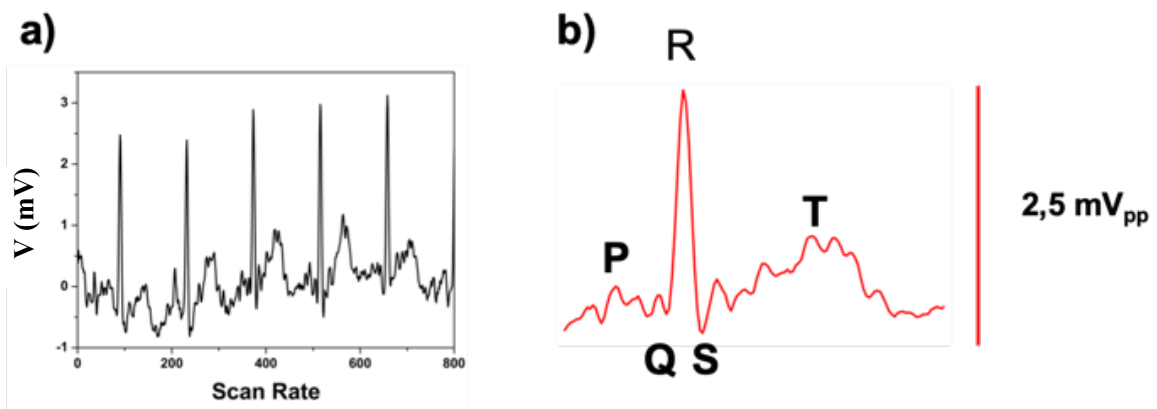


Figure 5.15: In a) the real ECG signal recording with an all-Printed-OEET on a Fibroin substrate. In b) the magnification of ECG signal with a 2.5 mV_{pp} amplitude of the main peak QRS.

Hence our prototypes, even though there is plenty of room for improvement and optimization, are already well suited for monitoring ECG signals and are characterized by amplification with respect to the case of OECTs on Kapton, in fact, in this case the main peak QRS presents a $V_{pp} = 2,5$ mV (Fig.5.15b) that is higher respect the value of 1 mV peak to peak recorded by an OECT on Kapton. This enhanced amplification can be ascribed to the fibroin substrate of its conformability and self-adhesion on the skin, rather than to a different gate-to-channel ratio (which, on the contrary, would realize a lower transconductance, i.e. a weaker transduction capability; Figure 5.10b). Besides eliminating the presence of any gel or other chemicals, the developed self-adhesion is expected to improve the interface among the device active channel and the skin. We have to face the fact that also the noise is increased and makes the Q peak more difficult to discriminate from the noise itself. Work is in progress to improve the signal to noise quality of the signal by optimizing architecture and the electronics working also on the transconductance and the amplification capability of the devices that is by far not the best achievable. Even though our results will require a wider systematic series of devices and measurements to better qualify their final performance and characteristics, we believe that the data presented here already show the validity and promising features of our novel approach.

5.5 SF/PEDOT:PSS blend for 3D OECT

A already mentioned in Chapter 3 Fibroin is the most important core protein of the silk ²¹⁴ showing a series of interesting properties, such as biocompatibility, biodegradability as well as interesting mechanical properties, that, combined with the flexibility to develop different geometrical structures makes it widely used in organic bioelectronics, drug delivery and photonics (sponge, fibers, films, hydrogels) ^{179,192}. Recently, 3D composite structures have been developed in the form of sponges, endowed with high porosity, paving the way to make electronic devices, including transistors, with a 3D active channel showing a higher active surface area respect to planar channels.²⁷⁸

SF as insulator material has been used as a dielectric layer in thin film transistor (TFT).²⁷⁹

We have hence explored the idea of using fibroin to prepare conductive blends that would maintain at the best properties of fibroin or that, at least, will keep them partially.

We hence developed a process to prepare a new composite made of SF and PEDOT:PSS. We aimed at a composite that would show the mechanical properties of the SF and the conductivity of PEDOT:PSS. The SF/PEDOT:PSS composite was prepared in the form of a 3D blend to fabricate a 3D channel of an OECT device.

As already discussed in Chapter 4 we first followed the process introduced by Rockwood et al ²⁰⁴, and hence the fibroin in aqueous solution was prepared dissolving the degummed fibroin in a 9.3 M

LiBr solution for 4 h at 60°C in a conventional oven. We then were dialyzed against ultrapure water for 48 h and centrifuged twice at 9000 rpm for 20 min. An 8% wt solution was achieved that was then mixed with PEDOT:PSS in 1:1 v/v ratio and stirred to induce a phase separation. The blend SF/PEDOT:PSS is separated from the solvent and segregated on the bottom of the vial, while the upper part of the whole products is constituted by the solvent (water) plus the excess of PEDOT:PSS unbound to the fibroin. The procedure used to bind SF with the conductive polymer PEDOT:PSS represents a typical procedure used in textile industry to dye fibers by acid dyes. Upon using acid dyes, ionic bonds are created between silk fibroin and the same dyes. At the isoelectric point (pH=4) ²⁸⁰ silk fibroin includes neutral NH₂ groups. Below the isoelectric point, acid dyes lets the NH₂ groups to be protonated in the cationic form NH₃⁺. ^{281,282}

Figure 5.16 shows the SF/PEDOT:PSS sponge after drying process in oven at 50 °C for 30 min.



Figure 5.16: Optical image of SF/PEDOT:PSS blend

The internal chemical bond and structure of the pristine silk fibroin, pristine PEDOT:PSS and the SF/PEDOT:PSS blend has been analyzed by Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) in the range between 1700 and 1450 cm⁻¹.

This range is commonly used to analyze the different structure of natural protein and it is associated with the absorption of the peptide backbones of Amide I (1700-1600 cm⁻¹) and the Amide II (1600-1500 cm⁻¹) which are the two major bands in the IR protein region. Amide I, strictly correlated to conformational backbone, generates the most intense absorption band and it is associated to the stretching vibration of C=O and C–N groups; the Amide II is associated to the N–H bending in the major part and stretching vibration of C–C and C–N groups. ^{283,284} Silk fibroin internal structure is assessed from the absorbance of the corresponding structure: the β-sheet peaks at about 1621,1520

and 1240 cm^{-1} , the random coils at about $1643, 1525$ and 1230 cm^{-1} and the α -helix around 1655 cm^{-1} .^{285,286,251} Figure 5.17 compare the ATR-FTIR spectrum of pristine silk fibroin, PEDOT:PSS and SF/PEDOT:PSS blend.

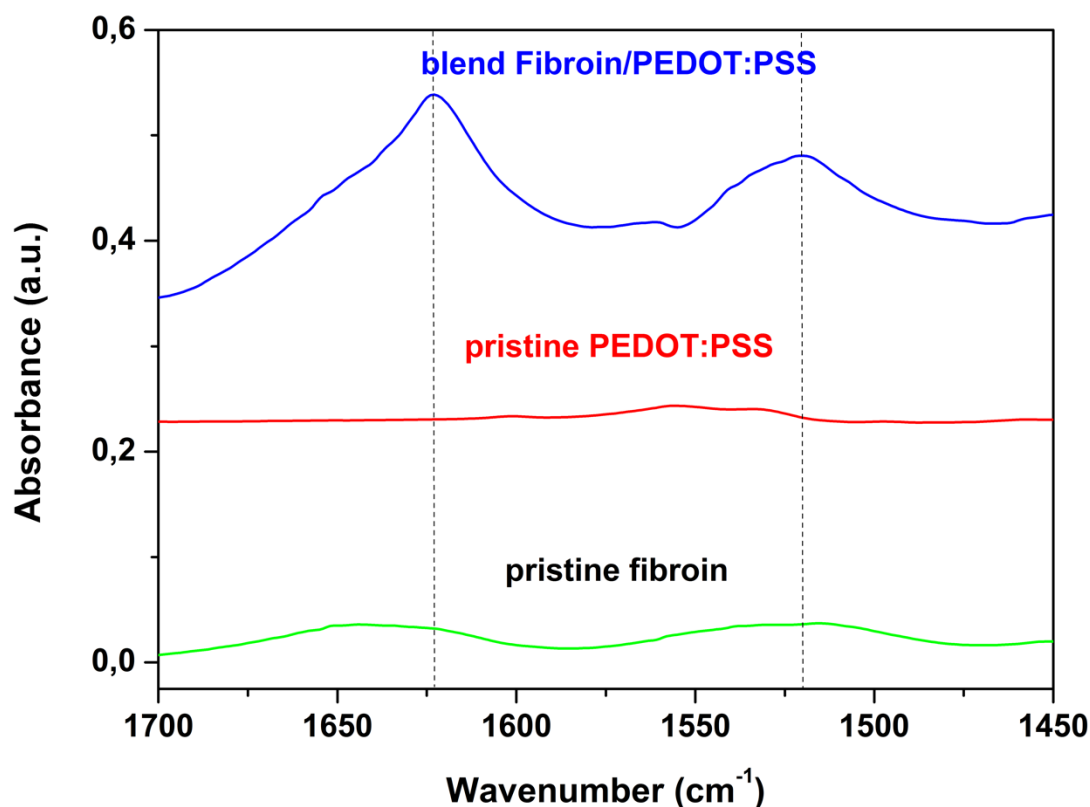


Figure 5.17: ATR-FTIR of pristine silk fibroin (green curve), Pristine PEDOT:PSS (red curve) and SF/PEDOT:PSS blend (blue curve).

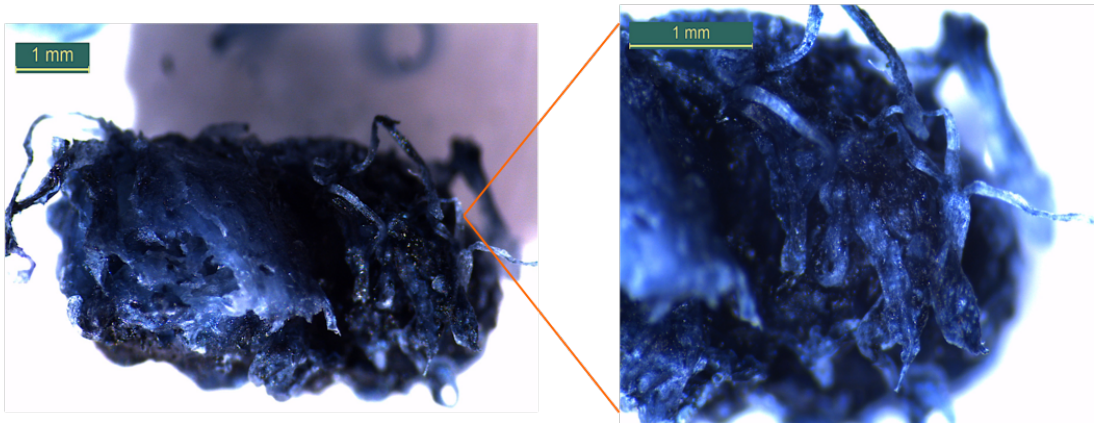
The pristine fibroin (green curve) presents two broad peaks around 1643 cm^{-1} and 1525 cm^{-1} , are assigned to a dominant amorphous structure, the main composition of the silk I.

The blend spectrum (blue curve) show two intense peaks around 1621 cm^{-1} and 1520 cm^{-1} , that are absent in the PEDOT:PSS curve (red curve); why are assigned in literature to β -sheet and amorphous structure, respectively. The adopted thermal process induces an enhancement of the β -sheet structure due to a rearrangement of the internal structure.¹⁹²

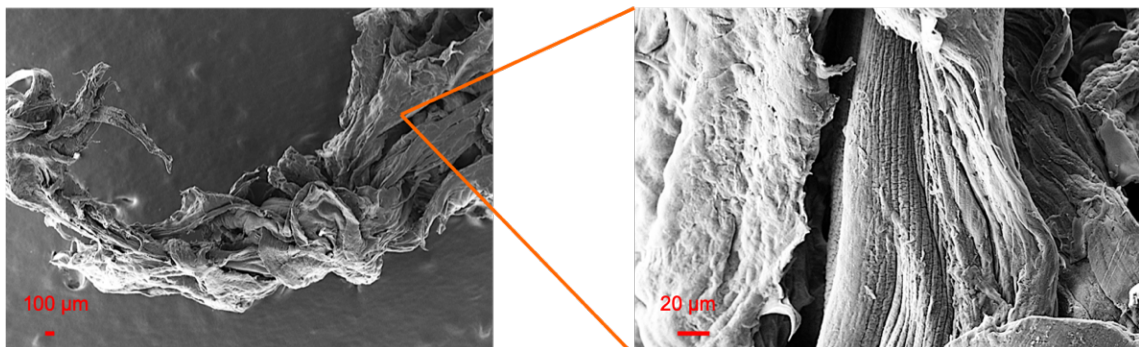
To investigate the morphology of the SF/PEDOT:PSS blend, optical microscopy and Scanning Electron Microscopy (SEM) were used Fig. 5.18 shows the corresponding images. A first macroscopic level of the porosity is observed in Fig. 5.18a, where the optical image shows a dense bundle of dark blue fibers interconnected to form a knotty 3D matrix (magnified in the left panel of Figure 5.18a). SEM micrograph add insight into the morphology of the blended material. The image

(Fig. 5.18b, left panel) shows silk fibroin fibers dyed with PEDOT:PSS, which are composed by several fibers in a hierarchical microstructure. This observed microstructure makes the synthesized SF/PEDOT:PSS blend highly porous, enhancing the active surface with respect to that of a pure PEDOT:PSS film, composed by a compact pancake structure. Under strain applied, the fibers show an elongation in the direction of the strain (Fig. 5.18c), demonstrating a quite relevant stretchability well suitable for applications in 3D flexible electronics.

a)



b)



c)

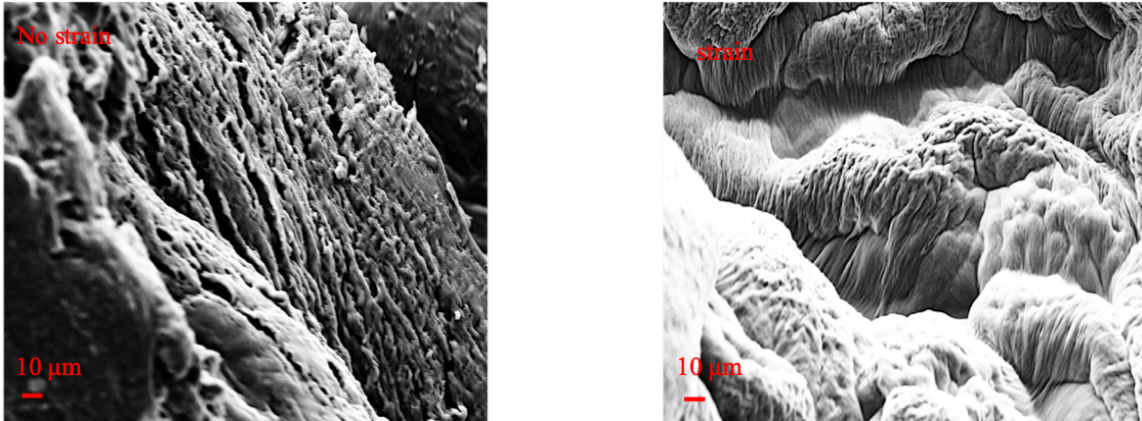


Figure 5.18: Hierarchical structure of SF/PEDOT:PSS blend has been confirmed by a) optical images and b) SEM images. c) SEM images show that the micro fiber follows the direction of strain applied.

The SF/PEDOT:PSS blend has been tested fabricating an OECT architecture the scheme of which is shown in Fig. 5.19a. The blend, in the gel form, was casted between the source and drain electrodes made of gold. The structure was then annealed at 50 °C to induce a 3D matrix formation. A PEO/NaCl 0,1 M solid electrolyte has hence been deposited over the OECT channel. A vertical Ag wire has been finally used as gate electrode in contact with the solid electrolyte.

Figure 5.19b shows the transfer curve of the OECT (I_{ds} vs V_{gs} at fixed $V_{ds} = -0,3$ V and by varying V_{gs} between 0 and 0,8 V, 0,01 V step).

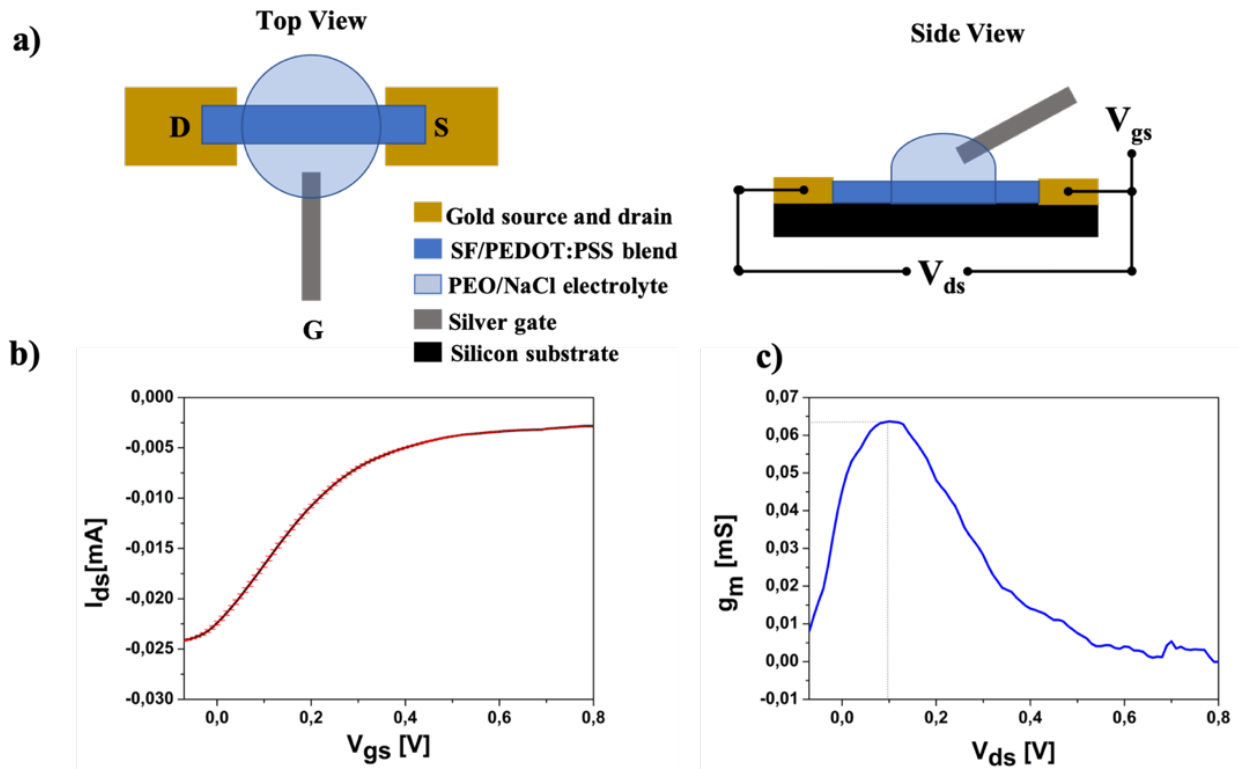


Figure 5.19: a) Schematics of the OEET based on PEDOT:PSS. b) transfer characteristics measured for $V_{ds} = -0.3$ V, and in c) the corresponding calculated transconductance, the maximum of which is centered in $V_{gs} = 0.1$ V.

The very low error bars (statistical average error, SAE, calculated as $\sigma / \sqrt{N}$, with $N=8$ number of measurements) are a good indication of the high reproducibility achieved for the OEET operation. The observed current modulation of the devices is comparable with that reported in literature for PEDOT:PSS stripes²⁵¹ and silk fibers dyed with this conductive polymer²⁸¹ even though is low compared to the pure PEDOT:PSS devices.

The maximum value found for transconductance was quite good $g_m = 0.06$ mS at $V_{gs} = 0.1$ V.

Since low operating voltages are desirable both in wearable electronics and for devices interfacing biological systems including human skin, the developed OEET with an 3D active channel made of the silk/PEDOT:PSS blend result to be a very promising candidate for applications in wearable and flexible electronics.

5.6 Fibroin thermal treatments

Fibroin optical and mechanical properties rely on its internal structure. As discussed in Chapter 4, after deposition fibroin is in the phase Silk I, where a high number of random coils and a small percentage of ordered structures (α -helix and β -sheet) are present. Several procedures are reported in literature, developing for inducing the transition Silk I/Silk II and hence achieving an increase of crystallinity. The ability to tune finely such internal modification and hence the material properties paves the way to a wide range of applications that become accessible, depending on the specific requirement that are needed from fibroin properties. The two typical fibroin treatments discussed in literature up to now, regard the methanol and water-annealing treatments. In the first case a dipping in methanol/ water solution induces a direct transition from Silk I to Silk II, while in the second case the synergic work of water vapor and temperature induces a slow transition between the two fibroin phases. During the PhD experimental activity, a novel method to promote the transition aiming at achieving a finely controlled tuning of the fibroin properties. In more details, the fibroin film, prepared from aqueous fibroin solution cast in a petri dish, has been annealed with a temperature ramp up to a determined temperature ($> 100\text{ }^{\circ}\text{C}$) and left at the desired temperature for 10 min. The crystal structure and modification induced by applying temperature have been evaluated by a wide angle X-Ray Diffraction (XRD) analysis, reported in Fig. 5.20

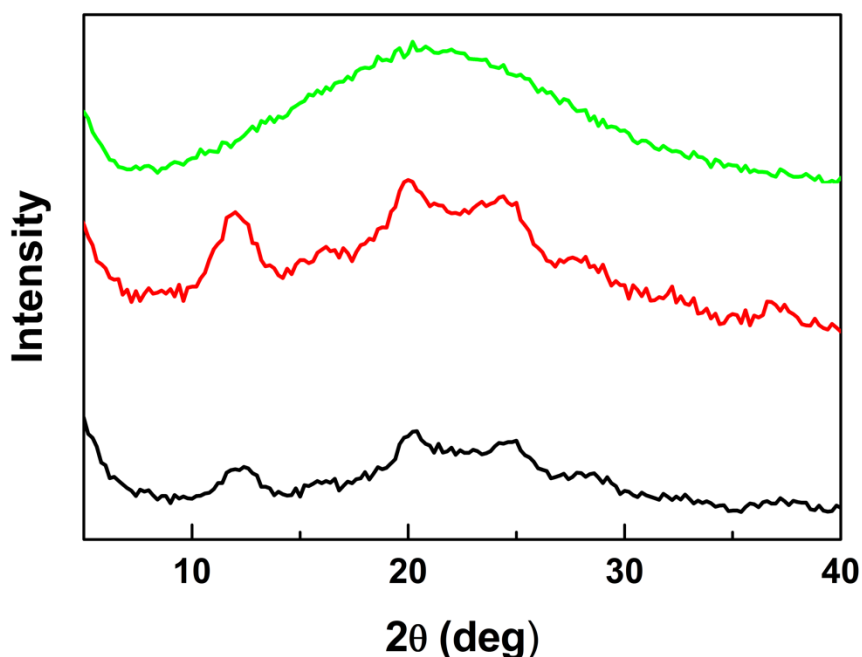


Figure 5.20: XRD spectra of pristine fibroin film (black line), silk fibroin film annealed at 175 °C for 10 min (red line) and silk fibroin annealed at 200 °C for 10 min (green line).

The XRD pattern of pristine silk fibroin (dark) shows the presence of the two ordered structures, α -helix and β -sheet, associated to the peaks at 11.95 ° and 24.02° for α -helix and 16.71 and 20.34 for β -sheet, respectively. After the thermal treatment at 175 °C (red line in the figure) an increase of the contribution from α -helix is observed, as shown by the induced increase of the peak intensity (11.95°). At the same time, also the contribution to the spectra of the β -sheets peaks increases, due to the rearrangement of the structure. For a thermal treatment at 200°C (green) the appearance of only a very broad peak demonstrates that over a certain temperature degradation of the fibroin ordered structures occurs that causes the presence of the single broad peak and the totally disappearance of any of the β -sheet and α -helix peaks.²⁸⁷ For this reason, in order to conduct the study described in the next section, the transition from Silk I to Silk II has been promoted by treating fibroin below 200°C.

5.7 High-Q silk fibroin Whispery Gallery Microresonator

As discussed in details in Chapter 3 SF has been used very successfully in a variety of photonic applications. Silk fibroin has been, in fact, used to develop and study several optical elements and optical devices, such as optical waveguide^{223,224}, microlenses²⁴⁰, diffraction gratings^{241,242}, lasers^{243,244}, light emitting transistors, inverse opals²⁴⁵ and Whispering Gallery Modes resonators (WGM)²²⁵. Since fibroin appears to be highly transparent, we decided to explore the use of silk fibroin in the visible spectral range of electromagnetic radiation, to implement a tunable refractive index system upon manufacturing structures with desired shapes and low roughness interfaces.

The main topic of this specific project, carried out during the research period at FORTH-IELS institute in Greece, was focused on the study of silk fibroin photoelastic properties, whose evaluation is still missing in dedicated literature. To achieve this aim we have chosen Whispering Gallery Modes (WGMs) cavities made of fibroin.

Whispering Gallery Modes was introduced for the first time by Lord Rayleigh in the 19th century with the aim of describing the phenomena of the whispering gallery, where sound waves remained confined in a resonator located under the St. Paul's cathedral in London.²⁸⁸ In a few words and quite schematically Whispering Gallery Modes are specific resonances or modes of a wave field (sound waves, electromagnetic waves) that remains confined in a circular pathway, inside a resonator (cavity) with smooth edges, due to a continuous total internal reflection at the cavity surface. These resonances strongly depend on the geometry of the cavity. Indeed, several shapes supporting whispering gallery modes have been proposed in literature.²⁸⁹

This work is focused on microresonators, or microcavities, that support optical frequencies of the electromagnetic field.

Several geometries have been reported such as microspheres, microcylinders, microtoroids (the first WGM cavity with fibroin) and bottle-shape optical micro-resonators. The latter are the cavities prepared and characterized in this work.

To prepare the WGMs cavities, silk fibroin was grown on a single mode optical fiber to prepare a micro bottle-shaped resonator. As already discussed, the fibroin aqueous solution was prepared using the method reported by Rockwood et al²⁰⁴.

To achieve a good confinement of the radiation, symmetric and thick cavities without any collapse in the middle are needed. For this reason, there are two problems to overcome; the first one is the high hydrophobicity of the optical fiber surface and the second is due to the coffee stain effect ²⁹⁰ during silk fibroin drying out, as this effect provokes a collapse in the middle region of the cavity.

We used an UV-laser treatment shined on the surface of the waveguide through a double long pass mask to create a pattern made of an alternating series of hydrophobic/hydrophilic regions, aiming at confining the drop of deposited silk solution (Figure 5.21a) in the as designed by the laser hydrophilic regions. A single drop of silk solution (5 μ L) has been hence casted with a syringe on the fiber surface and left to spread on the surface; in this way the hydrophobic regions tend to confine and push the drop in the hydrophilic part, allowing to avoid cavity asymmetry.

In addition, several optical fibers differing in terms of their inner diameter (80 – 10 μ m) have been tested to avoid the above described collapse of the fabricated cavity. The silk fibroin begins the drying process in the middle region, where a bubble air is formed during the evaporation process. This produces the formation of a thin layer of the material that tends to collapse. Using fibers with a small diameter, we could surround them by a higher content of material, creating in this way a thicker and more stable middle region, as reported in Figure 5.21b

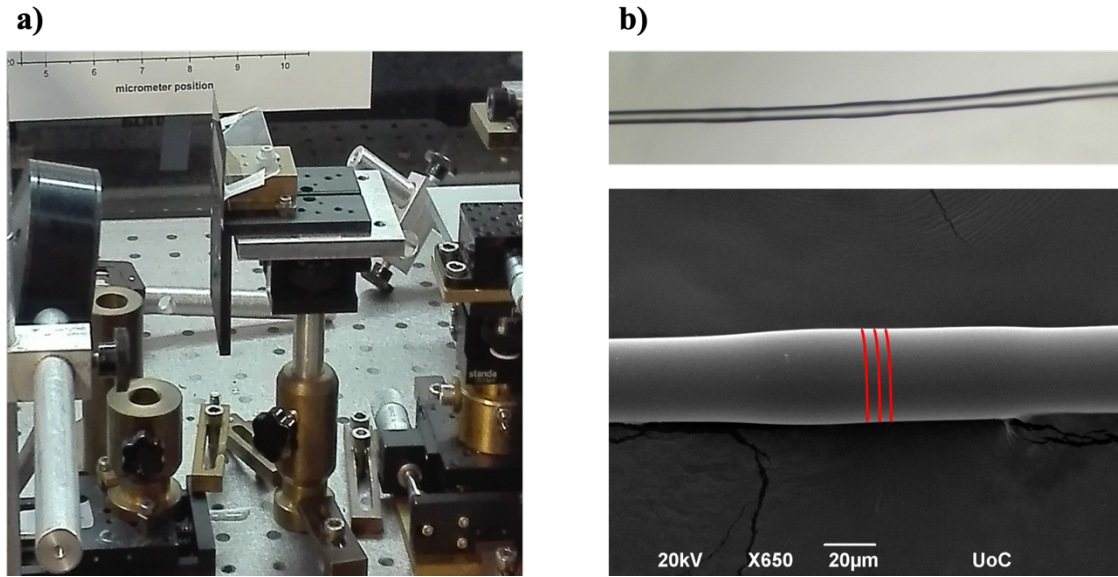


Figure 5.21: a) UV-Laser irradiation set up used to prepare the hydrophobic/hydrophilic pattern on the optical fiber surface. b) optical image of the cavity series on the optical fiber and the SEM image of the cavity ($34\ \mu\text{m}$) grown on a $30\ \mu\text{m}$ tapered optical fiber. The red lines show the light confined in the resonator.

For a practical application of WGMs cavities, an efficient and controllable coupling with the light is crucial. There are many ways to couple the light with the resonator that can be divided into three main categories: free wave coupling, fluorescent coupling and evanescent coupling, the one used in this work. Evanescent coupling occurs when a structure generating or guiding an evanescent field is put in contact with a resonator. The evanescent field of the resonator and of the coupler are made overlapping, so that the wave field travels from the coupler to the resonator and back.

A very efficient evanescent coupling occurs when a tapered fiber (i.e. an optical fiber that has a thinner diameter with respect to the original one upon heating and/or stretching) is used as coupler. This is because more light from the coupler can be caught by the resonator, thanks to the thinner diameter. This implies that the amount of evanescent field inside the resonator is a function of the fiber taper diameter. Nevertheless, if the evanescent field is intense, the high and low order modes are simultaneously excited and the transmission spectrum becomes more complex.²⁹¹ For this reason, we made an assessment of the optimal tapered diameters required.

Figure 5.22 shows a series of contact-coupling transmission spectra for tapered fibers of various thickness ($1,5\ \mu\text{m}$ to $2,5\ \mu\text{m}$).

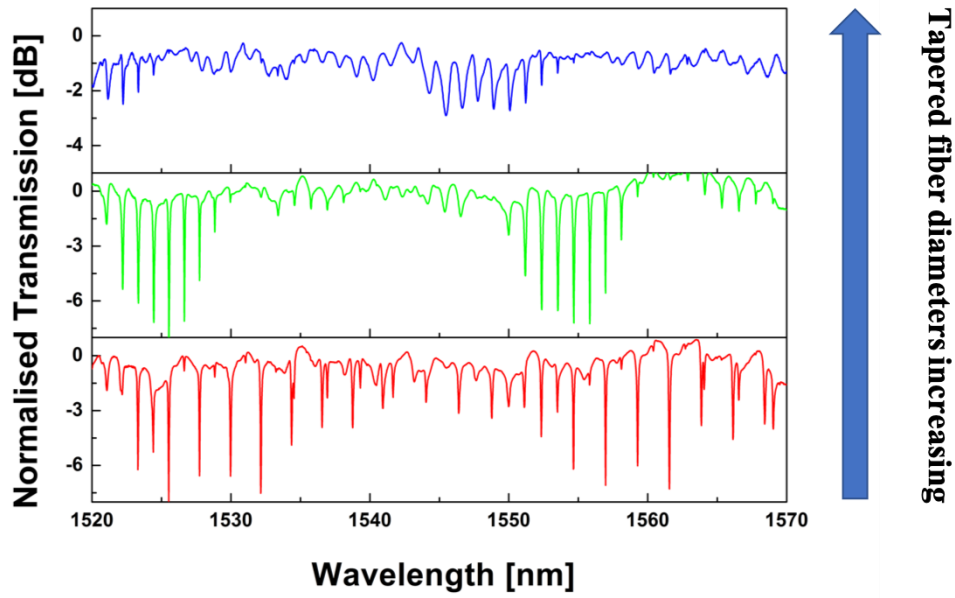


Figure 5.22: Transmission spectra for contact coupling of the resonator with different fiber diameters. From the bottom to the top, the tapered diameters increase moving the contact position with the resonator from 1,5 μm to 2.5 μm .

The number of resonance modes decreases when tapered fibers diameters increase, due to a reduction of the spatial extension of the evanescent field outside the coupling fiber. In fact, some Lorentzian peaks disappear and only high order modes remain in the transmission spectra, while if the tapered fiber becomes too thick, also the high order modes disappeared. Our analysis suggests that a diameter around 2.3 μm is the optimal compromise to reduce the low order modes and, at the same time, to achieve good peaks for high order modes. As described before, the quality of confinement inside the resonator is a function of the gap between the inner fiber diameters and the surrounding cavity extent. If the gap is too small, the light confined in the cavity can penetrate into the inner optical fiber and some silica modes can appear in the transmission spectrum, while if the gap is too high several defects (collapse, imperfections and so on) can appear in the cavity. For this reason, cavity growth on 30 μm inner fiber diameter (gap around 4 μm) has been selected and used for all the WGMs measurements. One of the most important parameters describing the performances of a resonator is the quality factor, or Q-Factor, defined by Eq. 5.4

$$Q = \frac{\lambda_0}{\Delta\lambda_{FWHM}} \quad \text{Eq. 5.4}$$

where FWHM is the linewidth of the wavelength (full-width-at-half-maximum FWHM) at the resonant peak and λ_0 is the peak wavelength at the resonance. This value describes the total amount of light that remains entrapped in the resonator due to internal refractions. It also provides information about the total losses of the cavity. High Q-Factor values correspond to a low amount of losses inside the resonator, indicating low roughness of the cavity walls, a good geometry for the cavity and low transmitted light outside the resonator. For example silica microresonators have high Q-Factors (best record values of 10^{10}).²⁹²

Another important parameter is the free spectral range, FSR, that is defined by equation Eq.5.5:

$$FSR = \frac{\lambda^0}{2\pi n(\lambda)R} \quad \text{Eq. 5.5}$$

The FSR describes the distance between two resonant peaks. FSR depends essentially from the kind of material, ($n(\lambda)$ is the refractive index of the material used to prepare the cavities) and from the cavity geometry (the radius, R).

As reported in the materials and methods chapter, the WGM cavity has been mounted on a homemade fork and coupled with a 2,3 μm tapered optical fiber that has been aligned with a polarizing fiber able to change the light polarization: in this way, transverse electrical (TE) and transverse magnetic (TM) modes of the WGM cavity have been excited separately.

Fig.5.23 shows the transmission spectra for the TE and TM modes acquired with an optical spectrum analyzer in a wide range of frequencies, from 1535 nm to 1585 nm and centered at 1560 nm. The TE and TM spectra (Fig. 5.23a and 5.23b) show a series of resonant peaks characterized by a small change in shape and intensity when the polarization of the light has been rotated of about 90 °. In addition, a blue shift between the TE main resonant peaks and the TM peaks has been observed due to the intrinsic birefringence of the pristine silk fibroin material.

A Free Spectral Range of 15,88 nm can be extracted by scanning a wide range of wavelengths (from 1520 nm to 1585 nm). This value is in agreement with the theoretical one (FSR=15 nm) for a cavity with a diameter of 34 μm and by considering a refractive index of the fibroin of about 1,55 at 1560 nm.

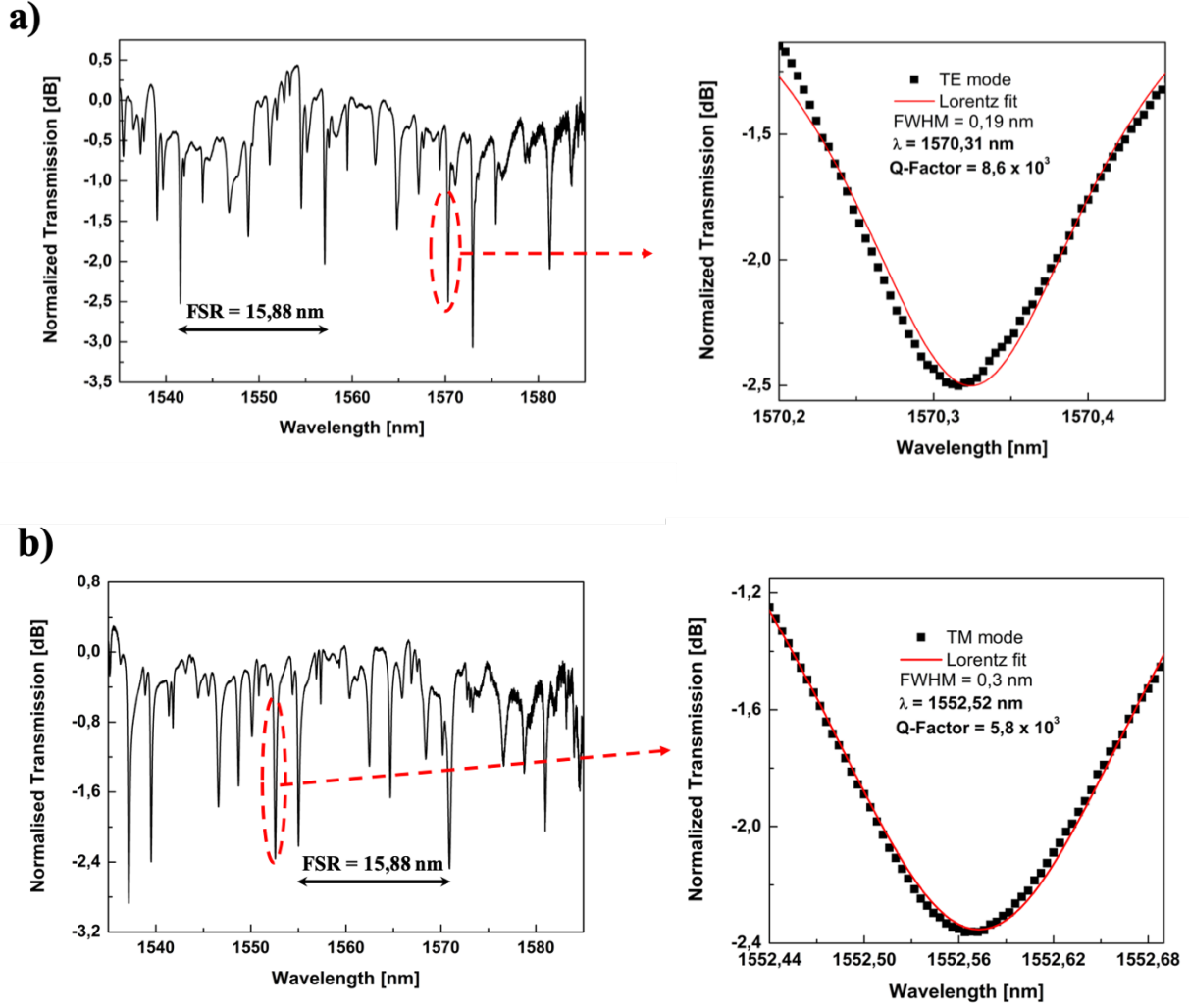


Figure 5.23: a) the WGMs transmission spectra for the TE modes for Silk Fibroin I. The Lorentzian fit of the resonance peak has been used to calculate the FWHM and the Q-Factor that are 0,19 nm and $8,6 \times 10^3$ respectively. b) WGMs transmission spectra for the TM modes. The Lorentzian fit of the resonance peak has been used to calculate the FWHM and the Q-Factor that are 0,3 nm and $5,8 \times 10^3$ respectively.

The Q-Factor, calculated by a Lorentzian fitting of the peak shape in the spectrum (red curve in Fig. 5.23a and 5.23b), is slightly less than of 10^4 for both the TE and TM mode. (Eq.5.4) These values are comparable with those reported in literature for other most commonly used polymers such as Polystyrene, PMMA and PDMS that (10^5 , 10^3 and 10^6 , respectively ^{248,293,294}).

As discussed in Chapter 3, silk fibroin can change his internal structure and the degree of crystallinity with the several processes described previously, one of these being the thermal treatment. Hence, a transition from Silk I to Silk II has been induced by means of a thermal treatment of the same microcavities characterized previously. The cavity has hence been annealed in a homemade furnace

at 180°C for 10 min; after cooling down, the new TE and TM transmission spectra have been collected in the same range and with the same set up used before.

Fig.5.24 shows the transmission spectra for TE and TM modes acquired after the annealing process.

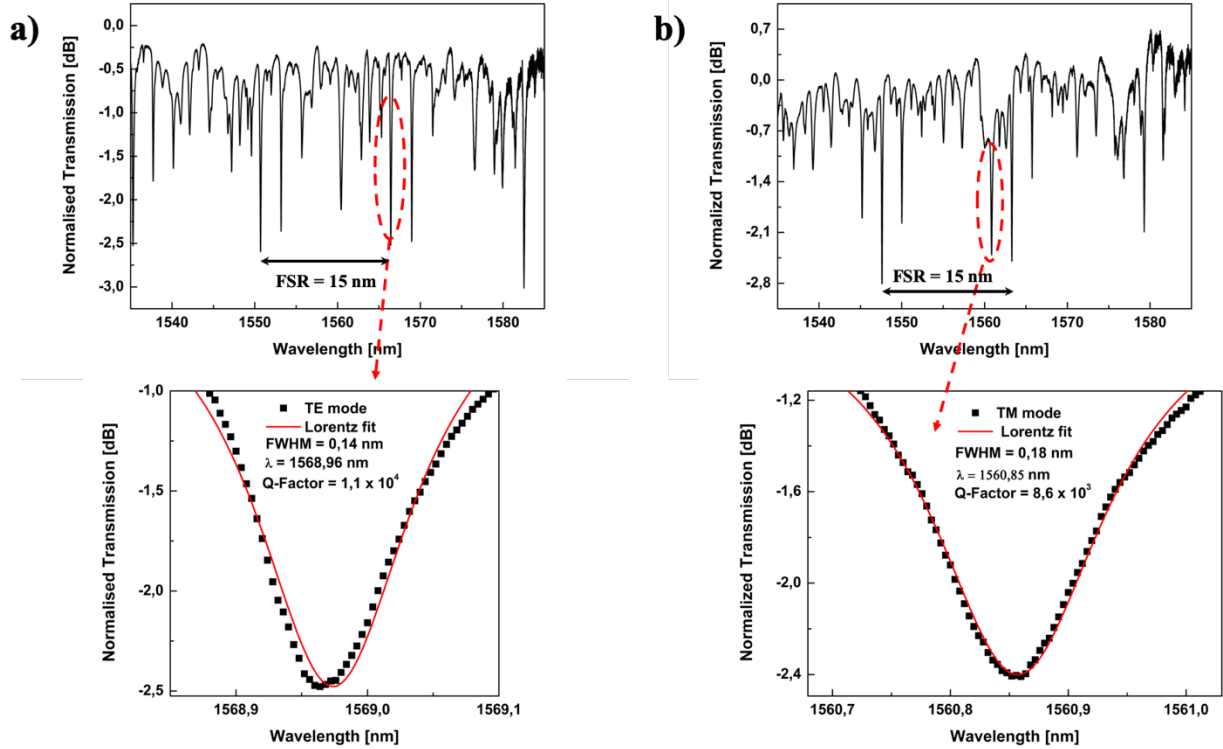


Figure 5.24: a) Silk II, WGMs transmission spectra for the TE modes. The Lorentzian fit of the resonance peak has been used to calculate the FWHM and the Q-Factor obtaining 0,14 nm and $1,1 \times 10^4$. B) Silk II, WGMs transmission spectra for the TM modes. The Lorentzian fit has been used to calculate the FWHM and the Q-Factor obtaining 0,18 nm and $8,6 \times 10^3$ respectively.

The transition from the Silk I to the Silk II generates an increase in the Q-Factor for the TE and TM modes, due to an increase in the crystallinity and hence the enhancement of the quality of the cavity. The fabrication of fibroin based WGMs on an inner fiber makes possible to study directly the effect of an applied strain on the resonator in terms of the presence possible changes of intrinsic material's properties upon application of the strain. The cavity is mounted on a homemade fork and by means of a motorized micrometer screw, some micro strains have been applied from 0 to 650 $\mu\epsilon$ for the TE and TM modes.

Fig. 5.25 shows the transmission spectra for the TE and TM modes acquired for a Silk I and Silk II cavities respectively.

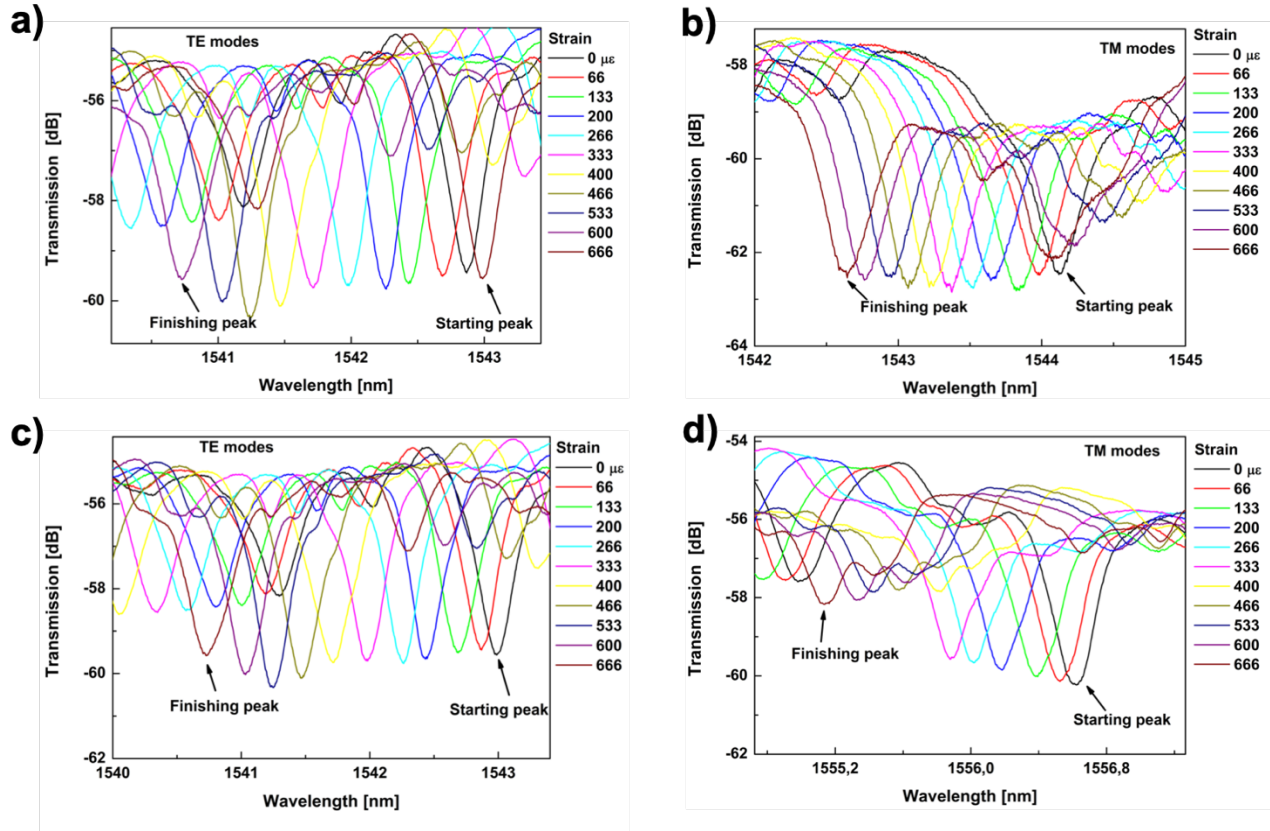


Figure 5.25: a) and b) transmission spectra for TE and TM modes respectively in a Silk I upon axial tensile strain applied. c) and d) the same analysis for the Silk II cavity.

A blue shift in the resonance peaks has been observed for both the TE and TM modes under external strains. This wavelength tuning as a function of the applied strain has been predicted by a simple theoretical model based on two fundamental assumptions ²⁹⁵, i.e. the dependence of the cavity diameter and the material refractive index from the amount strain applied, as described by the Eq. 5.5

$$\frac{\Delta\lambda}{\lambda} = \frac{\Delta n}{n} + \frac{\Delta D}{D} \quad \text{Eq. 5.5}$$

$$\frac{\Delta D}{D} = -\sigma \frac{\Delta L}{L} \quad \text{Eq. 5.6}$$

$$\frac{\Delta n}{n} = -P_{eff} \frac{\Delta L}{L} \quad \text{Eq. 5.7}$$

where $\Delta D/D$ is the fractional change in the resonator diameter (D) due to the deformation induced by the strain along the axial direction $\Delta L/L$ (Eq. 5.6), $\Delta n/n$ is the fractional change in the refractive index (n) induced by the applied strain (Eq. 5.7), σ is the Poisson ratio, P_{eff} is the effective strain-optic coefficient. The above equations predict that, by applying a strain along the WGM resonator a change in diameter and in the refractive index must be expected. In addition, these changes generate a shift in the resonance wavelength, as reported by Eq. 5.5. The negative sign shows that applied strain on the WGM resonator generates a blue shift like that observed in our case.

The measured wavelength shifts have been reported as a function of the applied strain. Fig.5.26 shows the response of Silk I and Silk II cavities to the applied strain for both the TE and TM modes.

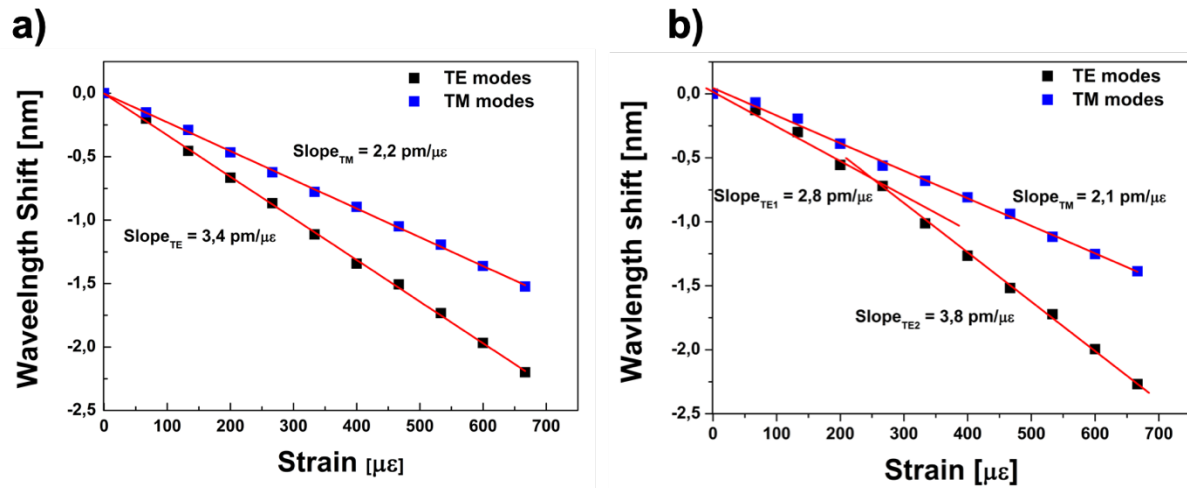


Figure 5.26: a) comparison of TE and TM wavelength shift for Silk I as a function of the applied strain the sensitivity of the fibroin is shown. b) sensitivity change in TE modes after thermal treatment

For each 2 μm of axial elongation strain a wavelength shift of 0.2 and 0.15 nm on average for the TE and TM modes respectively has been found (Fig. 5.26a). After the thermal process (Fig. 5.26b), an increase of about 0.1 nm for TE and of 0.05 nm for TM in wavelength shift has been observed under the same elongation. These results indicate that the WGM can act as a very efficient strain sensors. It is possible to calculate the sensitivity to strain for the TE and TM modes, but also for the two forms of fibroin. The wavelength of the Silk I WGMs resonance for both the polarizations (Fig.5.26a) exhibits a linear dependence upon the applied strain. The slopes calculated by linear fits indicate a sensitivity of the material to the strain equal to $3.4 \text{ pm}/\mu\epsilon$ and $2.2 \text{ pm}/\mu\epsilon$ for TE and TM modes respectively. Silk I show two different strain sensitivities for the two polarizations due to the inherent birefringence of fibroin. These values are better with respect to those of $0.64 \text{ pm}/\mu\epsilon$ of silica WGM

microresonator²⁹⁵ and they fall in the range of values found for polymeric cavities²⁴⁸. After annealing, the increase of β -sheets amounts inside the bulk of fibroin induced a change in the sensitivity upon strain. The average slope (Fig.5.26b) for the TM mode is almost the same found in the case of the Silk I, but the situation changes quite significantly in the case of the TE modes. Under a low value of strain (around 250 $\mu\epsilon$) the sensitivity value is comparable to that found for the TM, 2.8 pm/ $\mu\epsilon$ and 2.1 pm/ $\mu\epsilon$ respectively. Over this limit of strain, an increase of sensitivity has been achieved due to a higher change in the refractive index and, probably, due to a particular reorientation of the crystallites occurring along the strain direction. The strain sensitivity achieved in this case is 3,8 pm/ $\mu\epsilon$ which is much better than for other polymers (for example for PMMA 0,63 pm/ $\mu\epsilon$ ²⁹⁵) These results indicate that a new type of material, i.e. the fibroin, can be used as an on demand birefringent material for optical sensing and biosensing, whose properties are finely tunable upon applying external strains. Starting from these results, it possible calculate for the first time the photoelastic properties of fibroin. The assessment of photoelastic properties by silk fibroin are fundamental for its implementation on skin-attachable optical sensors. This evaluation is still in progress.

Conclusions and Outlook

In the framework of the fast developing research field of the printed organic electronics, this work explores, develops and studies printed and flexible electrochemical devices based on organic components aiming at novel strategies toward sensing, biosensing and *in vivo* bio signal monitoring developed on innovative and promising substrates. We started from the idea that direct writing techniques for fabricating electrochemical transistors and their sensing applications could fully take advantage of the advantageous features are demonstrating in several fields of research and in technological applications. Our research work and the results accomplished in this thesis deal mostly with Organic Electrochemical Transistors that are one of the most popular and successful devices for biosensing and for interfacing biological systems in particular because of the low operating voltage (< 1 V), the great transduction efficiency of ionic into electronic signals because of the gain that they provide together with the transduction. In our research work we worked at combining these precious advantages with the fabrication of devices on flexible and conformable substrates by direct writing techniques drawing high definition functional features designed and developed for advances in sensing and bio signal monitoring.

Aerosol jet printing technique (AJP) has been selected as the writing process best suited for our aims. We could in fact work with several kinds of inks, inorganic and organic, over a wide range of viscosity achieving high definition features achieving the desired miniaturization of the devices demonstrating their efficiency, unobtrusively and potentially wearable. We have been explored for the materials of our interest on achieving an optimal printing windows, to get a good control of the printing process and a high definition of the features used to miniaturize the printed electronic devices.

We hence have carried out, for the first time a comprehensive and systematic study, the results of which are discussed in this thesis, to find the optimal condition for PEDOT:PSS, the polymer of choice for our aims, direct writing achieving the optimal printed line with a reduced amount of imperfection and ill-defined parts that would shoot down the film quality and device performance. This is an important contribution to the field of organic electronics, because of the relevance of PEDOT:PSS as semiconductor material of choice and for the large number of applications that can be now explored by the efficient and reliable printability demonstrated here. On the other hand, a very important focus of our work has been devoted on exploring substrate that would innovate in sensing applications and device performance for monitoring bio signal directly on the human body. We developed a series of different OECT on different substrates including KAPTON, a very robust

and flexible substrate used widely in electronics but not yet significantly used as a substrate for printing PEDOT:PSS. We demonstrate in this thesis that we could achieve state of the art electronic performance by our optimized printing processes. Among the several substrates explored, we devoted large efforts to fibroin, an innovative and promising material of natural bio-origin for flexible and wearable electronics. We hence have developed, for the first time, a new procedure to deposit OECTs components directly on fibroin using the aerosol jet printing technique. We demonstrated that Fibroin, as a substrate in direct writing, can improve devices flexibility, stretchability and the adhesion on the body making possible bio signals monitoring *in vivo* and real time without the need of other materials such as gels to interface the skin.

We believe that our work on aerosol jet printing paves the way to a fast OECT prototyping for uncountable applications. The robust recipes developed and optimized give a reliable tuning of the channel geometry (width, length and thickness) and hence of the performance of the fabricated devices with the flexibility to cover a wide range of applications with a good reproducibility as shown in the flexible OECT electrical characterization. In particular as far as ECG recording is concerned, the optimization of PEDOT:PSS printing has been a key factor to produce the right dimension to shoot down the impedance, the crucial issue for the on skin measurement. We hence achieved via the development of a flexible and conformable substrate made of fibroin the ideal combination to realized devices that we may consider as among the best promising for *in vivo* biological signal recording. In fact our devices thought, designed and developed as prototypes in this thesis project, shows state of the art performance compared to the commercial passive electrode,²⁹⁶ in the case of the OECT on Kapton, and increase with the OECT on fibroin (1 mV_{pp} and 2.5 mV_{pp} respectively) giving advantages in term of easy to handle, flexibility, biocompatibility and self-adhesion on skin.

These preliminary results pave the way to a wide research work aimed at optimizing and improving performance characterization and specific fields of application. We believe there is a lot of room for optimization including materials properties, device and system architecture and so on. In particular for the device itself we plan to make a systematic extended study of the geometry to find the optimal compromise between width, length and thickness of the channel to reduce the impedance and increase the amplification power of the device. Critical steps will concern the fabrication of flexible robust contacts and hence improve the reliability of the devices. Our approach based on the aerosol printing represents hence a great opportunity to rapidly develop a series of novel efficient and reliable devices compared to the more standard methods based on photo-lithographic processes. Furthermore, the processes developed for fibroin preparation and for device fabrication can be in principle scaled to large area or industrial fabrication because combine a user friendly and low cost extraction procedure

with a technique (Aerosol Jet Printing) that is being already proposed by the manufacturer as well suited for industrial developing at least on a scale of pilot plants.²⁵⁴

Summarizing, the major relevant results achieved in this thesis, concerning materials preparation, inks development, printing optimized processes, all printed device fabrication, have been aimed at developing and qualifying OECTs on fibroin as very promising all printed devices for biomedical applications.

We hence have shown the feasibility of fully biocompatible, cheap, easy to handle and scalable systems that could contribute to the development of wearable and all-day user electrophysiology sensors. We are working to further improve these devices increasing their sensitivity by working on the gain and noise suppression to directly monitor also other neural signals that are intrinsically faster and less intense than ECG.

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