

Surface modifications of metallic electrodes for the reduction of inflammatory response after implantation.

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I WOULD PREFER NOT TO

— Herman Melville
(*Bartelby, the scribe* – 1853)

A Manu.

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— Nathaniel Hawthorne
(*L'artiste du beau* – 1844)

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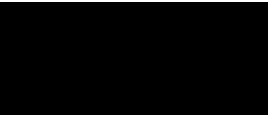
Abstract

Nerve implanted prostheses are of great interest for the recovery of damaged neurological functions by the electrical stimulation of the peripheral or central nervous system. The small size of the stimulation electrodes requires optimized electrical properties for sufficient recording/stimulating ability. This involves a close and long-lasting contact between the stimulation electrode and the biological tissues, weakened by the inflammatory reaction occurring after implantation. Improving this contact is one of the main challenges that must be faced in the development of long-term active implantable stimulators. Surface modifications of the materials intended for implantation are one of the keys evoked to control and improve the occurrence of the reactions to implantation, and reconcile both sides of the nerve/electrode interface.

In this thesis, we explore three types of surface modifications to address interfacial issues of stimulation electrodes. First, the physical (or structural) modification of the stimulation surface itself is performed through the creation of a model nano-structuration on the metallic surface, by a brush of self-standing metallic pillars of nanometric dimensions. The structures are observed and characterized, and the electrical properties of nano-featured electrodes are shown to be improved. Moreover, this model nanotopography is shown to have peculiar effects on cells adhesion, morphology and growth.

In the second chapter, we present the development of a method allowing the easy and reproducible fabrication of switchable conducting polymer-coated electrodes (polypyrrole) for the local and electrically-controlled delivery of anti-inflammatory dexamethasone molecules. Two techniques are investigated for the liberation of molecules and optimized for the control on the quantity liberated and the properties of the films, cyclic voltammetry and biphasic pulse potential pulses stimulation. Moreover, the development of a new device for the in-vivo stimulation of polypyrrole-coated metallic electrodes by biphasic potential pulses with determined parameters is evoked, and the first in-vivo experiments carried out.

Finally, we present a fabrication process of polypyrrole copolymeric films with specific chemical groups allowing further modifications. We demonstrate that the composition of the copolymer films based on modified pyrrole units can be tailored by playing on different independent electrosynthesis parameters. We also prove the possibility to synthesize multilayer polypyrrole structures for the simultaneous controlled delivery of molecules and grafting of biologically active groups at the surface of the outer layer.



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List of publications

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Leprince L., Dupont-Gillain C., Ferain E., Demoustier-Champagne S., Kalaskar M.D. 2012 Effects of model vertical nanopillars on osteoblast cells behaviour (submitted).

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A-CV	amperometric response in cyclic voltammetry
BS	benzenesulfonate
C_{dl}	double-layer capacitance
CE	counter electrode
CEP (or CP)	conducting electroactive polymer (or conductive polymer)
CV	cyclic voltammetry
DEX	dexamethasone (molecule or ion)
EIS	electrochemical impedance spectroscopy
EQCM	electrochemical quartz crystal microbalance
FES	functional electrical stimulation
G-CV	gravimetric response in cyclic voltammetry
HPLC	High-performance liquid chromatography
ICP	intrinsically conducting polymers
i_p	peak current
NA	nanopillar arrays
NWA	nanowire arrays
PC	polycarbonate
PNS	peripheral nervous system
PSS	polystyrene sulfonate
pTS	p-Toluenesulfonic acid (sodium salt)
PVD	physical vapor deposition
PVS	polyvinyl sulfonate
Py	pyrrole
Py-3-COOH (or Py-COOH)	modified pyrrole unit with a COOH group on position 3 carbon
PPy	polypyrrole
R	electrode resistance
RE	reference electrode
SCE	saturated calomel electrode
SI	stimulation electrodes
TS	toluene sulfonate
VNS	vagus nerve stimulation
WE	working electrode

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1 Introduction

THE WHOLE – MAN AND MACHINE TOGETHER – IS FAR MORE THAN THE SUM OF ITS PARTS

— Hunter Thompson
(*Hell's Angels* – 1966)

1.1 Objectives and strategies

Dysfunctions of the neural system is the main cause of a large number of pathologies which, to date, cannot be treated by classical surgical or medicinal therapies (Parkinson's disease, tetraplegia, paraplegia, hemiplegia, deafness, ...). An alternate solution spreading more and more since a few decades, and nowadays largely emerging in our everyday life, is the implantation of an autonomous device for the substitution, mimic or support of a failing human function. More specifically, the kind of device in the focus of this work is tailored for the electrical stimulation of reactive tissues, especially related to the nervous system (central and peripheral). In this case, one speaks about *neuro-prosthesis*.

Regarding recent examples on the interfacing of biomedical science with neural tissues, we can enlighten the successful development of nerve stimulation apparatus for hearing-loss, chronic pain, incontinence, erectile dysfunction, obesity and diabetes [1]. When speaking about research on implantable devices, scientists should keep in mind a long-term overview, following from the numerous requirements of official organizations (FDA, CE labeling, etc.). On electrical stimulation and recording techniques nowadays rely the diagnostic, therapeutic and treatment strategies for a wide range of neural problems. Furthermore, the accessibility to the neural system is growing concurrently to the understanding of its functioning. Communication with the central nervous system is more and more available through penetrating electrodes, to correct motor disorders for example [1]. The peripheral nervous system is also solicited in the control of pain, muscle contraction, and the activity of specific organs, translating failing brain process

messages into actions of external mechanical or electrical functions [2, 3].

A long story of device improvements coupled to engineering techniques lead to new perspectives in this field, at the meeting point of a lot of many domains, such as (micro-)electronic, biology, engineering, material science, etc. At present, the new outlook towards them is the use of nanotechnologies advancements, the foretell of a new era in bio-electronic devices.

Despite therapeutical successes and clinical applications, there exist fundamental challenges in the expansion of implantable stimulators. While surgical placement is at present under control, in the sense of less traumatizing for the implanted tissues, long-term electrode viability remains a significant problem [1, 4]. In fact, we observe a progressive loss of performances in stimulation or recording, concurrent and accordingly to inflammatory reactions resulting from their implantation, eventually seeing the appearance of a thick and resisting tissue encapsulating the nerve. This chain of reactions induces considerable morphological modifications within the direct or indirect environment of the tissues. The modulation of these reactions is considered the major challenge in the next years for the significative improvement of electro-physiological output of prosthesis.

In this context, the general aim of this thesis is to improve the interface between the implanted electrode and the biological tissues accordingly to the diminution or the suppression of the inflammatory reaction. Consequently, the long-term electro-physiological performances of the prosthesis should be guaranteed and even enhanced.

Some studies fulfilled in the present thesis are part of a project funded by *BioWin*, the health cluster of Wallonia. The *DEEP* project (*Drug-releasing electrode with enhanced properties*) is about the development of an implantable electrode with quantitative drug eluting capacity. Polypyrrole coating of platinum nanostructures forms the basis of this innovative technology. The consortium is composed of *Neurotech, SA, it4ip, S.A.* and research centers at the UCL (Louvain-la-Neuve): the *Institut de la matière condensée et des nanosciences - IMCN* and the *Institut de recherche expérimentale et clinique - IREC*; and at the ULG (Liège): *Département of clinical sciences*.

The main reported long-term limitations of electrode performances are due to charge transfer hindrance and impedance increase of the electrodes, in addition to the accrue distance between the stimulation electrode and the nerve to stimulate. A lot of efforts have been made in the design of electrodes, leading to the developpement of less traumatizing and more stimulation-selective implanted devices. Even if they are not yet commercialized, self-sizing cuff electrodes, which engulf the entire circumference of the nerve and contain metallic stimulation contacts, have been shown to improve the long-term nerve-stimulation performances. However, some impediments remain, related to the inflammatory reaction of the implanted tissue.

Surface modifications of the materials intended for implantation are one of the keys evoked to control and improve the occurrence of the reactions to implantation, and reconcile both

sides of the nerve/electrode interface. The enhancement of biocompatibility for implanted materials also comes with the reduction of macrophage adhesion onto the implants, especially onto the surfaces designed for stimulation. Three types of modifications are emerging in the recent developments. These modifications can be distinguished one from each other, but are usually applied as a combination, intentionally or not.

The first way to address interfacial issues is the physical (or structural) modification of the stimulation surface itself. Biological systems have the precocious ability to recognize particular features. At the molecular scale, surfaces are rarely flat, firstly as a result of their fabrication process. This topography (or roughness) has been shown to play a major role in the cell adhesion and bounding to the surface.

Literature about direct influence of topography (especially at a nanoscale) and the particular effects of a restraint number of parameters on cell behaviour (as a first step within the road to implantation) is wide and furnished. Nevertheless, it is even actually not possible to understand clearly the importance of each parameter, on one hand because of its interlacing with other modifications and on the other hand because of the - actually decreasing, but still great - difficulty to create model structures at a nanoscale. A first noticeable fact is that the reaction of cells to peculiar topographies depends on the scale of it, *ie* micron sized topography does not engender the same reaction as nanoscale topography.

Though chemistry and topography interact in a complex synergetic manner, it has been shown that topography by itself is able to influence the behaviour of cells in contact [2, 3, 5–7]. Giving the fact that implants are exposed to complex biological environments, they are in contact with multiple cell types. Those different cell types may not respond in the same way to the designed surface cues, and the optimization of implant surfaces will head to different levels of topography. Over the past few years, the fabrication of substrates of micro- and nanometer scales which replicate the structure and length scale of certain native topographies in two-dimensions have been created following the developments in advanced engineering techniques [6]. Cell responses to topographical cues depend upon a lot of factors : cell type, topographic geometry and size, physical properties of substrate, etc [3]. Rapidly growing literature conveys a lot of studies carried out about the effects of nanotopography, with a specific interest on cell morphology, attachment and adhesion, proliferation and migration, and complex biological responses such as genotypic alteration, differentiation or cell-cell interaction. The exact understanding of the connection between nanoscale surface designs and cell response is still not transparent.

More than the effect on cell adhesion and growth, the use of the peculiar properties of nanostructures is of major interest for the improvement of stimulation performances. As a response to their small size, the increase of active area has been widely used to enhance electrodes efficiency. One promising way to improve electrode performances stands in the creation of vertically aligned conducting nano-objects. The common designation for this type of structures is *nanowire or nanopillar arrays* consisting in vertically aligned nano-scale cylinders with a defined density, distribution, shape and geometry.

In the scope of this thesis, we focused on the fabrication of nanopillar arrays and on their influences on electrical properties in a first time, and on their effect on the adhesion, morphology and growth of osteoblast cells in short term in vitro culture, as a model for further investigation on cell interactions with nanoscale structures. This is the object of the first chapter.

Compared to literature, the originality of this work lies in the development of an easy and controllable structuration at the nanoscale, geometrical parameters being independently addressed. Moreover, the nanostructuration step can be integrated directly in the fabrication process of an implantable cuff electrode device, because all the procedures are compatible with the materials in presence.

The second biomaterial strategy currently explored to minimize or eliminate the undesired early inflammatory tissue reaction following implantation and to maintain signal transmission quality over time is the delivery of anti-inflammatory biomolecules in the vicinity of the implant.

One of the drugs commonly used for its intense anti-inflammatory activity is the dexamethasone (DEX), a synthetic glucocorticoid hormone. Systemic injections of DEX were shown to reduce tissue reaction around the implant in the central nervous system [8, 9], but this kind of DEX administration is very expensive and can cause serious side effects, such as myopathy and diabetes [10, 11].

To overcome these problems, local delivery of drugs by the implant itself is a promising and more effective strategy. Passive drug delivery systems were developed, based on simple drug diffusion, but did not allow the full control of the released drug doses. Therefore, there is currently a growing interest in developing electrically controlled drug delivery systems using electroactive conducting polymers as coatings. Indeed, due to their unique redox properties that allow controlled ionic transport through the polymeric film, these polymers have been considered for drug delivery applications.

Among conjugated polymers, polypyrrole (PPy) is particularly attractive because of its easy and efficient electrosynthesis from aqueous solutions, biocompatibility, good environmental stability and low voltage actuation [4, 12]. In its oxidized form, polypyrrole is doped and electrically conductive. This doping is reversible, which allow it to deliver in a controlled way the inside doping molecules. Literature reports on the interests of a coating with conjugated polymers on metallic electrodes for the use in stimulation electrodes. Up to now, PPy has already been studied for controlled delivery of different biomolecules, such as adenosine 5'-triphosphate (ATP) [13], neurotrophin [14, 15] and dexamethasone [16]. However, several problems were highlighted, in particular chemical and mechanical instability of the polymer. Improvements are still to be made, especially on the incorporation and release of biomolecules (particularly of large size), and above all on the reduction of the impedance at the electrode interface, in the aim of an efficient signal transmission and recording without the hazard of injury to the surrounding tissues induced by high currents. This is the object of the second chapter.

The novelty of this work can be found in the controlled delivery of anti-inflammatory molecules by polypyrrole using completely in vivo-compatible techniques and parameters in terms of the

integrity of both tissue and device. There is thus a direct possibility of in-vivo transfer and application of this drug liberation technique. Moreover, this study contributes to the understanding of electrochemical parameters effects on anti-inflammatory molecules release by polypyrrole.

The third and final solution to improve electrode performances by surface modification is the variation of the chemical composition. There exists a large variety of procedures for the modification of the chemical properties of the surface: biological or not, hydrophilicity modification, adsorption of peptides and proteins, etc [17]. Literature abound with examples of surface chemical modifications with cell growth promoting molecules (RGD peptides, ATP, DNA, etc.) [17]. For the previously explained reasons, polypyrrole is a premium choice for stimulation electrode coatings. However, in spite of all these advantages, PPy suffers from the absence of any functional groups that can be used for biomolecules surface modification. As biologically tailoring the surface of biomaterials is highly desired to further enhance their performance in many biomedical applications, a lot of efforts have been done in recent years for elaborating functionalized PPy derivatives through either modification of the pyrrole monomers structure or through preparation of copolymers [18–28]. For applications requiring good electroactive properties of the film, copolymers are however preferred to pure substituted pyrrole monomers due to the modification of the pyrrole ring which affects the electrical conductivity. Even though many functionalized PPy surfaces were successfully prepared, scarce investigations have been performed regarding the detailed electrochemical copolymer synthesis. In this aim, the investigation for a well-controlled electro-copolymerization in aqueous solution of pyrrole (Py) and pyrrole-3-carboxylic acid (Py-COOH) on flat gold surfaces was conducted. More precisely, the effects of the composition of the initial comonomer solution, the applied potential and the charge density passed for film formation, on the final degree of functionalization of the copolymer films were studied. Aside from that, the copolymerization electrosynthesis process was also adapted for preparing functionalized PPy nano-containers, presenting potential applications in drug delivery. Moreover, the possibility was explored on films with a combined effect of an external layer of functionalized copolymer film with an inner layer containing biologically interesting molecules. In literature, some interactions between those two explored solutions were pointed out. Among them, we can raise the concomitant limitations induced by the application of current on a functionalized structure. The latter forced researchers to be innovative as well as cautious in the purpose of the concurrent improvements brought by the complex assembly on one electrode. The fabrication of tailored surfaces for the functionalization with molecules of biological interest and the possibility to implement them on a drug delivery system is the object of the third chapter.

In opposition to what was observed in literature, a new approach to modify the surface chemistry of stimulation electrodes with biomolecules is developed in this chapter, which takes into account the electroactive nature of the device. This chemical modification can thus be added to other surface modifications and considered in applications requiring good electrical conductivity.

Chapter 1. Introduction

The characterization of novel stimulation electrode surface modifications is the main objective of this work, in the outlook of improving long-term performances. This fundamental analysis takes into consideration the limitations induced by an eventual in-vivo application. In spite of this, the natural continuation of this process is on one side the combination of the three studied surface modification approaches, and on the other side their implementation. As preliminary results, some in-vivo experiments are shown at the end of this work, a preview of the future work. The context of the present work and an abstract of each surface modification studied in this thesis are summarized in figure 1.1. The next section presents in more details the objectives of each chapter of the present work.

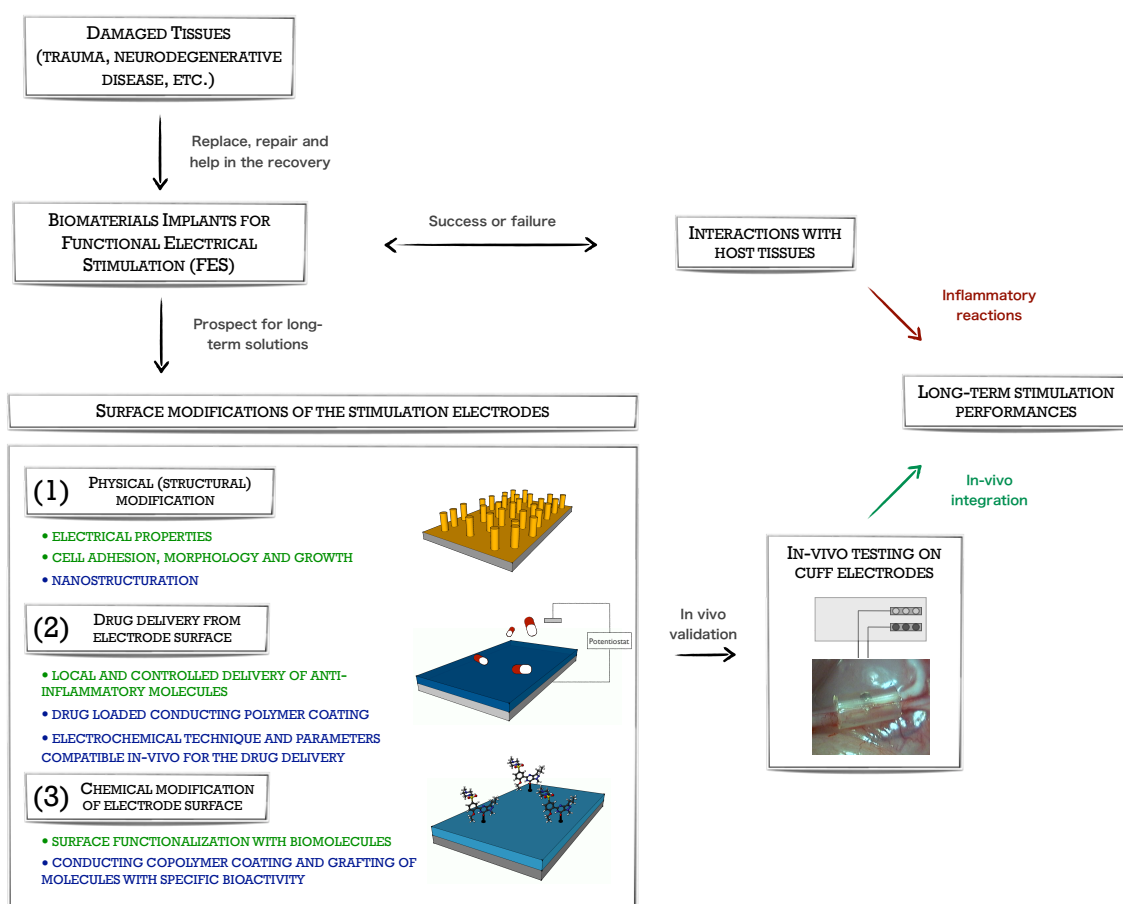


Figure 1.1: Schematic representation of the context and objectives of this thesis.

1.2 Outline of the thesis

Starting from this general context and strategy, specific objectives can be drawn for each part of this thesis. Primary, it will begin with a detailed overview of the state of the art relating to the general and particular concepts used for the accomplishment of this work. The results and discussion relative to the present work will be separated in three chapters.

The first chapter reports on the development of a fabrication technique of nanowire arrays with controlled dimensions and their effects on the adhesion, morphology and growth of osteoblasts cells. The aims of this chapter may be summarized as follows:

- Fabrication by an electrochemical hard-templating method of nanopillar arrays with controlled dimensions (diameter, height and density) ;
- Characterization of the electrodes structure and electrochemical properties ;
- Analysis of the importance of nanopillars on osteoblast cells behaviour in short term in vitro culture in terms of adhesion, morphology and growth.

The second chapter describes the elaboration of a conducting polymer coating doped with anti-inflammatory molecules and the local and controlled delivery of them (in time and quantity) by electrochemical techniques compatible with a future in-vivo application. The aims of this chapter may be summarized as follows:

- Elaboration of flat and nanostructured electrodes covered with dexamethasone-doped polypyrrole films with controlled characteristics ;
- Analysis of the dexamethasone delivery from polypyrrole by electrochemical techniques (cyclic voltammetry and biphasic potential pulse stimulation) with the influence of parameters on the liberation profile and quantity ;
- Development of a device for the in-vivo liberation of dexamethasone from polypyrrole films synthesized on cuff electrodes by biphasic potential pulses.

The third chapter depicts the elaboration of a copolymer film with modified polypyrrole units for the further surface modification by the grafting of a molecule of particular biological interest. The possibility for the integration of such film as the outer layer of a drug delivery system is also assessed. The aims of this chapter may be summarized as follows:

- Electrosynthesis of thin copolymer films based on carboxylic-acid modified pyrrole units and surface covalent modification with a protein-repellent polymer (poly(ethylene glycol) chains) ;
- Analysis of the effect of electrosynthesis parameters on the degree of functionalization (comonomer solution, applied potential and charge density) ;
- Electrosynthesis of thin copolymer films as outer layers of doped polypyrrole coated electrodes and investigation of the effect of the functionalized copolymer film on drug delivery and inversely.

Finally, this thesis is concluded by a brief summary of the main results and the perspectives of the present work, with a special outlook on in-vivo applications.

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2 State of the art

WITHOUT LITERATURE, LIFE IS HELL.
— Charles Bukowski

2.1 Functional Electrical Stimulation

2.1.1 Historical discovery and development of functional electrical stimulation

The use of electricity through natural electrical phenomena is reported since the High Antiquity of Egypt [1], as certified by low-reliefs (ex. sepulchre of Ti in Saqqarah, 2750 acn). Although the mechanism was unknown, the treatment imposed by these ichthyic electric shocks has been used by physicians of the Roman Empire as a remedy for migraines or the *gout*. The discovery of electricity and the ways to store and supply it go hand in hand with the development of electrotherapy, and more precisely of electrophysiology. The latter is described as the study of the electrochemical phenomena occurring in the cells of living organisms, and in particular in neurons and muscular fibers [1]. At the biological level, neuroanatomy made possible the study of neuronal sub-cellular protrusions and the cells supporting them. It is worth noticing the incredibly precise description of the peripheral nerves microarchitecture around this period.

In 1791, Luigi Galvani developed the phenomenon called *galvanic element*, whereby nerves could be stimulated to provoke muscular contraction in frog legs. His interpretation of the phenomenon is analogous to the one for torpedo fish electric organ (which is a modified muscle), as the discharge in the metal of the electrical energy contained in the muscle. Alessandro Volta demonstrated in 1792 that this galvanic element used electricity. Nerves since are considered as the electric wiring of the body.

The expansion of the utilization of electric currents in medicine appeared in the first part of the nineteenth century, thanks to the *static machines* (or *friction machines*) and the use of capacitance to obtain high potentials. Physicists, biologists and botanists therefore largely exploit these machines for the research in electrophysiology, but with varying degrees of success and enthusiasm (see figure 2.1).

The discoveries in the middle of 19th century of static electricity, capacitance and electromagnetic inductance aroused the curiosity of physiologists on muscular contraction phenomena, nervous conduction and cell excitation. This resulted in the proposal, by Johannes Muller, of the *law of specific nerve energy*: *the kind of sensation, following the stimulation of a sensory nerve, depends not on the mode of stimulation but on the nature of the sense organ with which the nerve is linked* [2]. While T.H. Huxley generalized the idea that the central nervous system is the regulator of the body (in its famous *Manual of the anatomy of invertebrate animals* [3]), the foundations of what will become the modern use of electrical stimulation (*aka Functional Electrical Stimulation (FES)*) were laid.

Even if at this time, the medical applications of electricity were numerous, the underlying principles were not fully understood. The twentieth century saw the arising of technological advances in electronic and computer science, finally leading to the development of modern applications of FES, as for example miniaturized integrated circuits. A large number of modern applications are now fully controlled and widespread in the treatment of numerous diseases, as



Figure 2.1: Experiments on facial muscle contractions evoked in a patient by the local application of galvanic current. Guillaume Duchenne de Boulogne [4]

it will be presented further on.

The most representative example of technical achievement in the application of FES for the treatment of a disease is the cardiac pacemaker. At the same time, numerous other applications are successfully developed towards the long-term implantation of an autonomous device. We can mention the cochlear and ocular implants, the drop-foot stimulator, phrenic stimulator or vagus nerve stimulator [5]. Nowadays, the growing comprehension of the nervous functions combined with the important miniaturization of complex electronic devices allow researchers to rapidly implement new treatments based on functional electrical stimulation, with an extremely high specificity and reduced invasivity.

2.1.2 Principle of rehabilitation by functional electrical stimulation

Pathologies affecting the nervous system can be the source of an important number of diseases (Parkinson's disease, muscular paralysis, deafness, epilepsy, etc.). Most of these afflictions cannot be cured by the classical surgical or medicinal therapies. However, in some cases, functional electrical stimulation is proved to inhibit, modulate or induce a nervous message that results in the disappearing of those symptoms.

The general principle of FES is the application of small currents to a localized part of a nerve (or directly inside the central nervous system) to induce stimulation of the tissue in contact. Depending on the characteristics of the treatment (tissues, type of stimulation, etc.) stimulation parameters are adjusted. The stimulation parameters likely to be modified are the current intensity [mA], the stimulation frequency [Hz], the duration of the impulsion [μ s], and the duration of the stimulation cycles. In some cases, it is also possible to temporary inactivate the stimulator (and reactivate it afterwards), in order to block the secondary effects related to the stimulation.

In the scope of this thesis, vagus nerve stimulation is considered. Vagus nerve stimulation (VNS) was demonstrated to prevent epileptic seizures by sending regular, mild pulses of electrical energy to the brain via the vagus nerve, and is also applied to treatment-resistant depression. The electric pulses are supplied by an implanted device [6]. The treatment will not be explained in details here. In the next section, a brief description of the implanted stimulator for the performing of vagus nerve stimulation will be done.

2.2 Implantable devices

As explained by Hatton et al. [7] : *the VNS consists of a constant current pulse generator / stimulator, a single subcutaneously placed lead wire, and a silicone rubber-imbedded platinum electrode wrapped around the left vagus nerve. The combined pulse generator/stimulator delivers an electrical stimulation burst based on programmed parameters. The VNS does not monitor central nervous system electrical or peripheral muscle activity, and therefore, cannot respond to potential seizure activity.* (see figure 2.2).



Figure 2.2: Diagram of vagal nerve stimulator placement. Adapted from *Cyberonics, Inc.* [8]

How electrical stimulation of the vagus nerve modifies seizures risk in epileptic patients is not completely understood. Traditionally, the vagus nerve has been considered to be a parasympathetic efferent nerve regulating autonomic functions such as heart rate and gastric tone. Most researchers, believe that VNS electrical stimulation creates action potentials within the cervical vagus nerve that modulate cerebral neuronal excitability, either through activation of the limbic system, noradrenergic neurotransmitter systems, or generalized brainstem arousal systems [7]. This effect may be through induction or inhibition of electrical signals by altering neuronal electrical or chemical properties.

2.2.1 Stimulator

The vagus nerve stimulator is composed of three parts: a stimulator (microelectronic generator for the electric stimulation and recording, and the communication with the user), a thin flexible wire (lead) that sends mild stimulation to the left vagus nerve, and the electrode with multiple metallic contacts in direct contact with the nerve. Two types of commercial stimulators are presented in figure 2.3. The electrode is the most critical part of the device, because it is in direct contact with the living tissues. The requirements and limitations linked to this part will be explained in details in the next sections, as it is the main focus of this thesis.

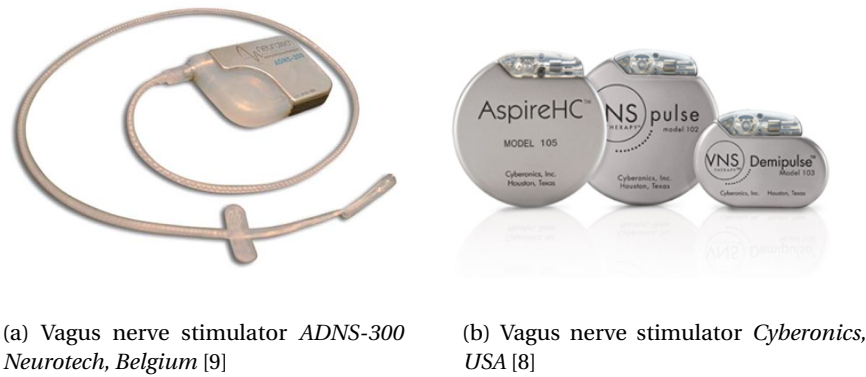


Figure 2.3: Implantable vagus nerve stimulator: microelectronic device.

2.2.2 Electrodes

Diseases and damages to the nervous system are particularly difficult to deal with in terms of consequences and possibilities of treatments. The scope of action for functional electrical stimulation have been widely described in literature and the obligation to interact through an electrically stimulating electrode pointed out. The interface with the most complex biological system is characterized by a high vulnerability to external stress or violence and genetic disturbance. Within this leeway, an *engineer-like* approach for designing electronic implants is often inappropriate (in the best case). It is usually worth remembering that nerves cannot be considered only as *electrical cables* that need to be connected to make some current pass. As borrowed to Heiduschka et al. [10], nerves are *long, sensitive structures consisting of biological membranes with multiple receptors and delicate interactive elements for online sensing the environment and transmitting information via molecules, potentials and changes in the chemo-electrical activity*. Based on these considerations, we can state that the principal requirements in designing a new implantable stimulation device is the integration of the system in its living environment by mimicking some biological functions of nerves and replacing them in the scope of implantation. On the peripheral nervous system (PNS), challenges on engineered devices surfaces are due

to unique biological three-dimensional structure of nerve cables. Informations conveyed to or recorded from the nerves are determined by the resolution from the fibers in a 3D structure. Selectivity of stimulation is defined by *the ability to activate one population of neurons without concomitant activation of another* [11]. Selectivity or specificity of signals evoked/recorded is dependent on the proximity of stimulation or recording [12]. Therefore, electrodes are best placed close to the nerve fibers that they should interact with.

Biological constraints to implantation involve tolerance to electrodes placed close to nerve fibers (and the number of them), distinction of single fiber signals (or their stimulation) in the bulk of the nerve from the surface. Damage to peripheral nerves can cause loss of function. It is thus critical to minimize implant-induced trauma. In summary, the main biological challenges associated with PNS interfacing can be described as followed : selectivity of stimulation, signal-to-noise ratio, implantation associated injury to nerve fibers, stability of the interface over time (inflammation) and collateral consequences (pain or sensory/motor triggering) due to physical movement or inflammation.

Implantations of electrodes for nerve stimulation and signal recording have been carried out for many years with great success, sometimes. Implantable devices must fulfill a certain number of requirements directed to the electrodes and the substrate which carries it. The later carries a few considerations in the scope of this field but it is out of focus of this thesis. As a summary of the type of electrodes in contact with the peripheral nerve system, Navarro et al. designed a table of electrode types in function of their selectivity and invasivity [12], as it can be seen on figure 2.4. The desired selectivity of stimulation or recording from individual nerve fibers or motor units increases in parallel with the invasivity of the electrode implantation, considering the application of different types of electrodes (despite that selectivity actually depends on the type of nerve and anatomical and physiological considerations for each particular application).

We reach at this point the core of the research. As they are directly in contact with the nerve of interest, on the implanted electrodes lie the achievement of the whole artificial system. At this point, we can enlighten a list of conditions for electrodes to present good performances. As a matter of clarity, we will from now talk about the whole implantable device which will be placed around the nerve as the *electrode* and the metallic part(s) of stimulation surface(s) as the *stimulation electrode(s) (SE)*.

In the next sections, we will develop the different aspects and considerations of electrodes design and characteristics at first. The limitations linked with implantation will next be evoked, mostly related to induced biological reactions. In a third time, the effect of stimulation electrodes characteristics (material, design, etc.) and the possibility to modify them so that device performances remain high will be analyzed.

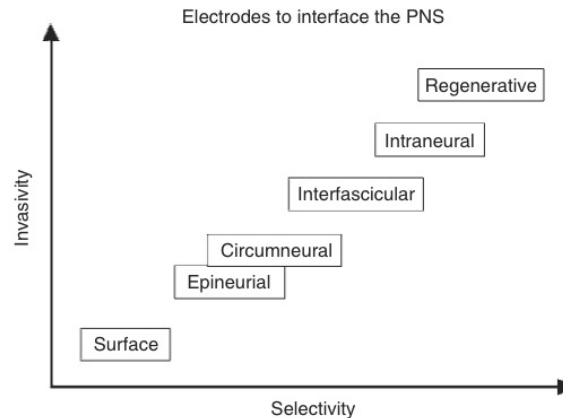


Figure 2.4: The different types of electrodes applied to interface peripheral nerves classified regarding invasivity and selectivity. This represents a general classification, despite that selectivity actually depends on the type of nerve and anatomical and physiological considerations for each particular application. Adapted from Navarro et al. [12].

2.2.2.1 General considerations and different electrode types

At this point, some general considerations can be stated. Nerve electrodes are implanted adjacent, around or even within a peripheral nerve trunk or spinal root. So, electrical stimulus intensity is reduced by this proximity to the nerve, in comparison with muscular electrodes for example. In consequence, side electrochemical processes and power consumption of the stimulator can be decreased [13]. However, nerve electrodes have the possibility to damage the tissues on which they are implanted. These considerations in addition to the ones on the nature of mechanical, chemical and electrical compatibilities thus require a wide study before a possible clinical application of a device [10].

As found in literature, there are currently a determinate number of **technical challenges** in the conception of efficient neural prostheses [14]. Electrodes first have to be sufficiently **small sized** to interact with a restrain number of neurons or even neurites. Reaching this goal is also related to the diminution of injury to the tissue and scarring, which will be evoked in the next section. While interacting with myelinated axons, the electrodes *radius of action* has to be sufficient to reach the nearest node of Ranvier. With small size comes optimized geometry and electrical properties, and high relative charge-transfer capacity. Consequently, electronic components have to be adapted : high signal to noise ratio, sufficient shielding between channels and against external interferences. To a larger degree, further miniaturization entail several difficulties related to the fundamental limits of currently used materials in terms of charge storage capacity and impedance. This level of reduction in size requires not only this development in new materials but also different methods of stimulation and recording.

The second challenge is linked to the **mechanical properties** of electrodes. There can be several

interpretations of mechanical properties related problems for implantable devices. In the scope of this work, we will consider mechanical properties primarily from the standpoint of long-term biocompatibility. As can be summarized in a more general consideration, the stability of materials in the electrode is crucial because, once implanted, it remains within the body of the patient for many years. Due to the insertion into the body and their conformation therein, mechanical stress can occur on the stimulation electrode surface or on the nerve. This limitation is particularly significant in the design of cuff electrodes. As a consequence, delamination of conductive interfacial layers have been reported for many types of metal electrodes, resulting in an increased inflammatory response (inflammatory tissue and protein deposition [14]). Even more damaging is the formation of a gap between the device and the neural tissue to stimulate. This gap is a barrier for recording and charge injection capabilities over time because of the presence of a layer of electrolyte between the conductive coating and the backing of the stimulation electrode. In addition, it is also important to remember that outer-layers materials must be insulating in order to avoid *cross-talking* between electrodes. In the great majorities of cases, a flexible material should be preferred to reduce mechanical damages.

The third and crucial challenge is linked with the complexity of the biological surrounding of the implanted electrode. The goal of every implantation is to achieve a **stable biology-tissue interface**. In other words, this stability is characterized by a minimal cellular change in the surrounding tissue and the continuous functioning of the implanted device. The development of stimulation devices is, indeed, currently limited by the drop of their electrical performances over extended periods, mainly due to an undesired inflammatory tissue reaction following the surgical implantation of the neural stimulator and their constant presence in the host tissue [15, 16]. An overview of the causes and the phenomena occurring during inflammatory response due to the implantation of neural stimulator will be done in the next section. As an outcome of the contact with biological fluids, enzymes and macrophages produced during the body reaction (as it will be explained in details later), electrodes have to be corrosion resistant (passively and when electrically stimulated). In literature, we can found a lot of studies of platinum, iridium, tungsten and stainless steel as stimulation electrodes and silicone elastomer, polytetrafluoroethylene, and polyimide as insulating carriers [10, 12, 17]. As well, numerous designs of electrodes have been reported. Literature reports that cuff electrodes are the most appropriate for vagal nerve implantation. The denomination *cuff electrodes* applies to the devices which engulf the entire circumference of the nerve and contain two or more electrode contacts exposed at their inner surface. They enable long-term stable recording/stimulation of mean activity resulting from summation of the activity across a bundle of active fibers within a nerve trunk.

In order to reduce the invasive character of the cuff electrode, the shape of these electrodes or of the array of stimulation electrodes has to be well-suited to the natural configuration and dimensions of neurons or axons, taking into account the vascularization at the site of implantation. These electrodes are constituted from insulating tubular sheath surrounding the nerve trunk (usually made of silicon). This insulating tubes have the important role to

facilitate peripheral nerve recording [18]. First, by ensuring all action currents to flow within the restricted volume conductor of the insulated nerve trunk rather than out of the nerve, increasing recorded signal amplitude. The longer the cuff, the less current leaking through the surrounding tissue and thus the higher the signal amplitudes [19]. Second, by protecting the inner activity recordings from external generated stimulus artifact signal. An optimal cuff length can be determined, as studied by Struijk et al. [20]. The predicted length is however too long to suit on a nerve. It is thus necessary to consider and assume a certain leak of current out of the device.

The stimulation electrodes are sandwiched between two insulating films and exposed at

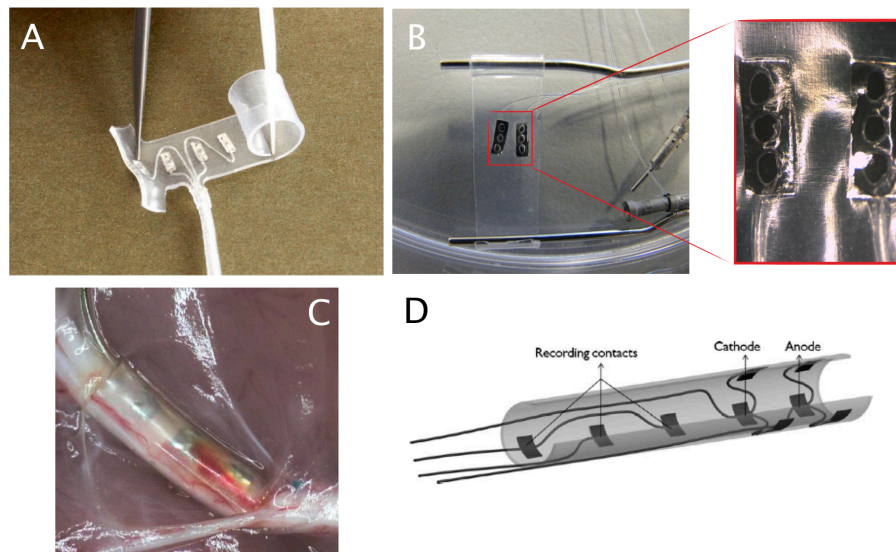


Figure 2.5: Cuff electrodes with multiple platinum contacts (A) Ardiem Medical [21] ; (B) UCL (GREN-MOEX) ; (C) Implanted on rat sciatic nerve ; (D) Cuff design from Thil et al. [22]

their inner surface (see figure 2.5). The connection to the stimulator is done via independent insulated wires (in order to address separately each stimulation electrode). Cuff electrodes have been fabricated in several different configurations, the most common being spiral cuffs or helix-shaped cuffs [9, 17, 19, 23]. Advantages of this type of electrode are numerous. They first allow the correct positioning of electrode leads, minimizing the mechanical distortion and eventual failure. The stimulating current is also confined to the inner space of the electrode, eluding side stimulation of other neighboring tissues and nerves [13]. It has been reported the decrease in required stimulus current of an order of magnitude versus other types of stimulation electrodes (ex. muscle). Applied preferentially at peripheral nerves, their implantation is relatively easy and the nerve is not damaged by proper surgical implantation. Despite a decade of successful implantations, the delicacy of nerve tissue and the physical properties of electrodes are sometimes not possible to combine. First historical models of cuff electrodes were stiff, resulting after implantation in damage of nerves due to mechanical displacements, nerve fiber or vascularization compression/stretching. Modern cuff electrodes tend to avoid this, using

flexible materials and compliant geometries (self-sizing electrodes). Regarding stimulation, old cuff designs did not have accurate measurement performances because recordings were more of superficial sum potentials. Axons in the center contributed less significantly to the measured signals and were in accordance less exposed to stimulation. Advanced designs of cuffs bear smaller electrodes disposed around the perimeter of the fiber bundle. This allows to address central axons as well. Accordingly, high selectivity of stimulation can be achieved via longitudinal or transversal currents by an appropriate control of stimulation electrodes switching [24].

From a functional point of view, simple cuff configurations are bipolar or tripolar (using central pole as cathode and two outer pole as anodes), which reduce current leaks out of the electrode. Multichannel electrodes enable selective stimulation of separate axonal fascicles within the same nerve. More recent implantations of several small cuffs around different fascicles or branches of nerves to selectively operate functional stimulation have been done. Stimulation methods have also been improved in order to circumscribe the region of excitation of the nerve trunk, significantly ameliorating selectivity. In addition, cuff fixation must be perfectly achieved otherwise the electrode may rotate around the nerve or shift along it, engendering a loss of efficiency and selectivity. Yet, the first prerequisite for the success of such complex electrode designs and stimulation protocols is a thorough knowledge of the distribution of nerve fibers within the nerve bundle and their function.

2.2.2.2 Stimulation electrodes: overview and limitations

In addition to the previous considerations about electrode characteristics, it has been evoked sooner in this section the crucial role of stimulation electrodes. Because they are in close contact with the tissue that they stimulate, the success or failure of tissue-interfacing devices depends precisely on the interaction between the living host and these synthetic materials which are used as stimulation implants. As aforementioned, on tissue-electrode interface relies the success of long-term implant performances. Electrical impulse is done through one or more stimulation electrodes distributed around the nerve trunk or fibers. These surfaces should have peculiar properties in order to ensure the best interface properties as possible.

Designed to support long-term functional restoration, neural stimulation electrodes performances are currently limited. Indeed, in vivo recording and stimulation slackens after a few weeks or months. Several mechanisms are established to entail the performance diminution. As described widely over literature, fibrous encapsulation of the implanted material is one of the main challenge to face in neural stimulation. Associated with foreign body reaction, fibrous encapsulation ends with the increase of impedance at electrode interface and accordingly the contraction of transfer signal effectiveness. This is a result of the so called inflammatory reaction of the body, following implantation of stimulation device. This sequence of biological reactions leading finally to the aforementioned consequence on electrode performances will be explained

in details in the hereunder section.

For the conductive materials composing electrodes, a wide variety can be found in literature such as gold, platinum, iridium oxide, glassy carbon, titanium nitride [25, 26]. Even though considered (and FDA approved) safe, typically fabricated smooth surfaces usually are not favorable to tissue integration. Release of ions from metallic electrodes is also a risk to affect tissue response. Essential considerations are the safe charge injection capacity above which hazard to the nerve can be done and the stimulation threshold of the surrounding neural tissue. For platinum electrodes, typical charge storage density is 6mC/cm^2 [27] and safe charge injection (in order to avoid platinum hydrolysis resulting in the release of toxic chemical species) is $100\text{--}250\text{mC/cm}^2$ [28].

Associated to the configuration of stimulation electrodes, the interface between metal (or any other conducting material) and the tissue to stimulate shows a significant fluid gap through which electrical signals must be transduced. From that statement, it is easy to figure out the significance of the distance from electrode to neural cells in the determination of current or charge threshold required for cell activation [29].

2.2.2.3 Inflammatory reaction

2.2.2.3.A Reactions following implant surgical placement The goal of every implant placement is to ensure a stable interface, ie a minimal cellular change in the surrounding tissue and constant long-term functioning of the implanted device. The handling of the host response is critical for implant stability as it can help to preserve neurons near the interface. The functionality of currently used neural electrodes is limited by the biological response resulting from surgical implantation, and their presence in the host tissue [22, 30–32]. The aim is to ensure the long-term interface stability from both points of view, device and nerve. We can distinguish different causes for the inflammatory reaction of the implanted nerve tissues and their consequences on both the electrode performances and biological response.

The most obvious and direct cause of the inflammatory reaction is the surgical implantation by itself. The trauma engendered by the placement of the device induces stress on the tissues and the inherent chain of reactions. The latter will be explained in the last part of this section. A second cause of nerve induced damage is the poor adaptation of electrodes to tissue fragility and dimensions. Connective tissue usually forms around an electrode and fills all available space. Loose fitting electrodes will consequently have thicker layers of connective tissue between the cuff and the nerve. Sustained mechanical irritation can furthermore induce injury to delicate capillary forming in connective tissues, which can exacerbate connective tissue thickening [33, 34]. To minimize the likelihood of sustained mechanical irritation, the cuff and the nerve should be bound closely together. According to Hoffer [35], the cuff should be at least 20% larger than the diameter of the nerve to prevent compression neuropathy caused by post-surgical oedema [36, 37]. Compression neuropathy affects the larger fibers most severely [38], but self-

spiraling cuff can help minimizing these effects [17, 23]. A lot of work has been done on the damage caused by the implantation of nerve cuff electrodes.

Consequences are observed on both biological tissues and electrodes. Tissue reaction considerations include trauma to axons as well as encapsulation of the implant in terms of nature and extent. The presence of nerve cuff induces connective tissue covering and changes in nerve shape so as to completely fill the space of the implant [39]. Traumatized axons may not conduct action potentials. Thick encapsulation increases the resistance and the distance between contact stimulation electrodes and the targeted axons and so degrades the electric field gradients. A local ischemia (loss of blood flow) is observed in nerve that receive cuff type electrodes. Typical histological signs of past ischemia trauma take the form of crescent shape regions containing thinly myelinated axons (believed to be re-myelinated axons) and greater axon distance occupied by connective tissue. A subsequent loss of conduction is observed [23] as a result of local ischemia. Conduction returns as axons are remyelinated.

Electrode properties are also modified as a consequence of encapsulation. Cuff electrodes are relatively stable in long-term recordings, even if stability is hard to quantify. However, modifications of electrical properties are observed. Large changes of impedance are reported after implantation [20, 40]. There is an initial decrease in impedance attributed to a local fluid accumulation between nerve and electrode resulting from an increase in vascular permeability in response to the surgical procedure [41]. However, this trend is inversed after a short time, and the initial amplitude of the recorded nerve decreases within a few days after implantation [40]. It is worth noting that there exists a strong linear relationship between nerve signal amplitude and electrode impedance. Thil et al. studied the time-course remodelling (morphological changes) and electrophysiology of the rat sciatic nerve after spiral cuff implantation [22]. They observed a relatively constant increase of impedance during the first month following surgical implantation. They attributed the low impedance and high rheobase¹ between implantation time and day 2 to an oedema induced by surgery. This will be detailed later in this section. As previously mentioned, when an implant is brought into the host body, there are a lot of events taking place, eventually leading to chronic inflammation, in the form of glial scarring and the replacement of neuronal with glial cells, loss of electrode function due to encapsulation [22, 30] and impediment to axonal re-growth [14].

As we have seen the general causes of inflammation resulting from the implantation of a cuff electrode, we will now discuss the consequences of them and the means of interactions with them. In order to act on those consequences, it is thus crucial to understand the biological chain of events taking place onto the electrode, eventually leading to the previously described disadvantages. Inflammatory reaction involves responses of a lot of actors, from cells to vascular system. Implantation induces modifications : fibrous encapsulation of the electrode, epineural thickening demyelination, axonal loss, initial axon degeneration with subsequent regeneration,

¹The rheobase reflects the fiber excitability and may vary with changes in the volume conductor or with axonal pathology. Chronaxie is the pulse duration required to trigger excitation with a current intensity equal to twice the rheobase.

intraneural growth of encapsulation tissue and vascular sequences (in [30], see figure 2.6). The

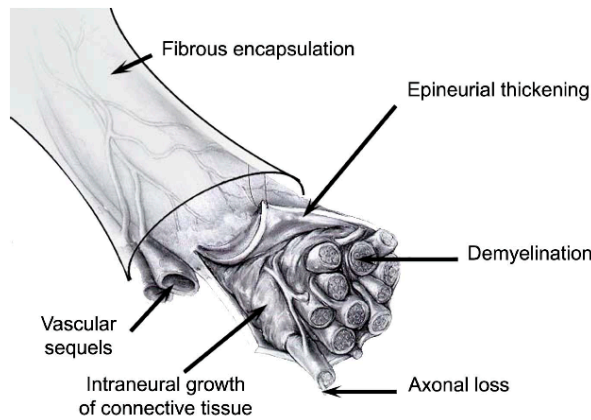


Figure 2.6: Morphological modifications after cuff electrode implantation. Adapted from Vince [32]

general sequence of events after implantation starts with an acute inflammatory response and leads in some cases to a chronic inflammatory response in a long-term view. Granulation tissue development can also be observed, as well as a foreign body reaction and fibrous capsule development [30]. A general sequence of events taking place in the process of implantation of a biomaterial, prostheses or medical devices can be seen in figure 2.7 A (summary) and B (sequence) [42].

As shown on the table (2.7 A), the initial inflammatory response is activated by injury to vascularized connective tissue, regardless of the tissue or organ onto which a biomaterial is implanted (blood-material interaction). Changes occur in vascular flow, caliber, and permeability and there happens the exudation process (fluid, proteins, and blood cells escaping from the vascular system into the injured tissue). The initial response of a material in a biological medium (implant) is the shielding by water molecules. This occurs in a time scale of a nanosecond. Surface properties of implants determine the pattern of interaction with water. This is also determinant on the proteins and other molecules that will adhere afterwards. Thus, when the implant is placed into the body, protein adsorption is the first consequence onto the surface of the implant. Several proteins can be found in relatively high concentration in biological fluids, depending on the biological environment of the implant. It will certainly be the major part of the first adsorption layer [43]. The details of this process is dependent on the surface of the implant as well as biological environment and nature of proteins. For example, adhesion of certain kinds of proteins (fibronectin, collagen) can favor adhesion of tissue cells [10, 44].

Even though electrode-tissue interface should be preserved, encapsulation of the implant by autologous material (protein layers, fibroblast, endothelial cells, etc) is needed, to favor integration and hide it from the immune system, and to a lower extent to diminish mechanical movement of it [10]. Nevertheless, it can also be a drawback by favoring the adhesion of bacteria and lead to infections. Provisional matrix development is the immediate consequence of injury

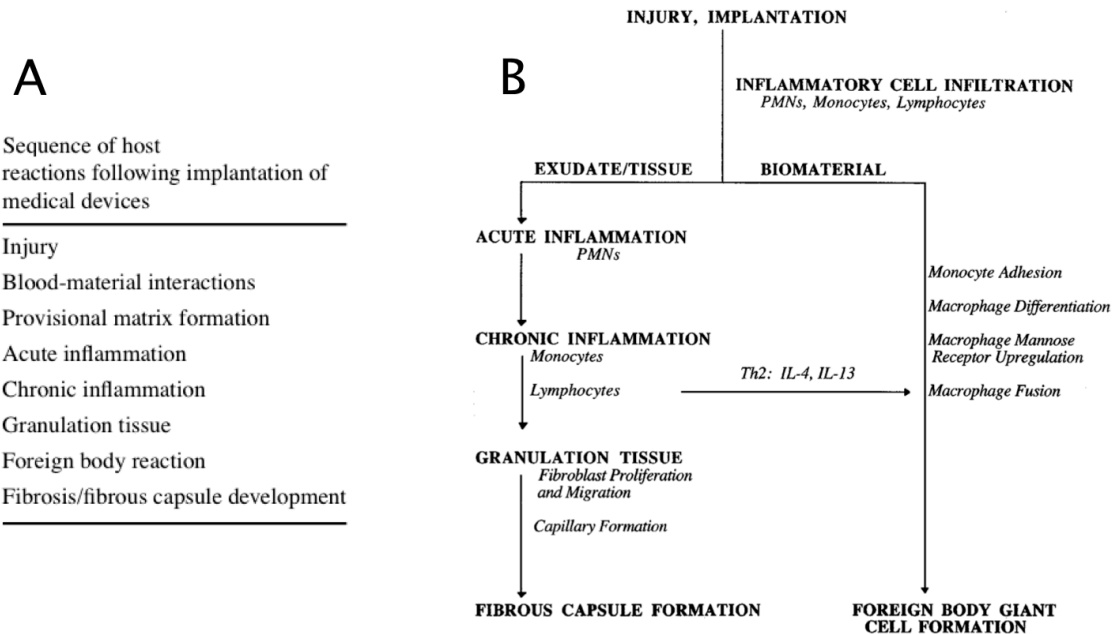


Figure 2.7: Summary (A) and sequence (B) of the inflammation steps following the implantation of a biomaterial. Adapted from Anderson et al. [42]

to vascularized tissue in the implantation procedure. This occurs within minutes to hours after implantation. Provisional matrix is composed of fibrin (produced by activation of the coagulation and thrombosis systems) and inflammatory products (activated platelets, inflammatory cells and endothelial cells) [42]. During this phase are initiated the resolution, reorganization and repair processes, by components within or released from the provisional matrix (*ie* fibrin network). Inflammatory cells and fibroblast recruitment begin at this point (cytokines (TGF- α , TGF- β , platelet factor 4, etc)). Provisional matrix can be considered as a natural sustained release system for the subsequent wound healing processes to happen. A general consideration in biomaterial study is the temporal variation of the inflammatory response in terms of the species in presence. It has classically been described in terms of the acute and chronic inflammatory responses and of the fibrous capsule formation that is seen over various time periods following implantation, as can be seen on figure 2.8.

The acute-phase response is characterized by changes induced by stress in the neuroendocrine and immune systems [10]. Concentrations of proteins from serum plasma either increase or decrease during acute-phase inflammatory reaction, depending on their type. The process consists in the migration of leucocytes from blood vessels walls to the implantation site which requires specific interactions mediated by integrins, selectins and immunoglobulins [45]. The result is the release of cytokines, as the principal mediator (and so one evaluation factor of inflammation). Cytokines are polypeptide hormones that regulate homeostasis in their tissue

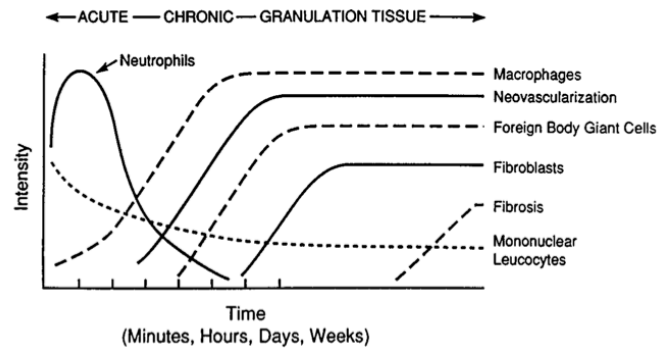


Figure 2.8: The temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, and foreign body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created in the implantation and the size, shape, topography, and chemical and physical properties of the biomaterial. Adapted from Anderson et al. [42]

of origin with an extremely low extracellular expression within the PNS as biologically active mediators. However, in specific cases, cytokine expression and activity are increased. A peak is observed in response to nerve injury or infection, and macrophages are the principal source of it. High levels of $\text{TNF-}\alpha$, IL-6, IL-1 and IFN- γ are observed. Interactions are thus between cells and the surface-bound proteins, for hours to days after implantation. At this stage, adhesion, migration and differentiation of cells occur. Important parameters are at this point : proteins in particular and more generally biological molecules, the released products from the material and its microstructure [46]. The permanence of inflammatory stimuli lead to chronic inflammation. Chronic inflammation is less uniform histologically than is acute inflammation. In general, it is characterized by the presence of macrophages, monocytes, and lymphocytes, with the proliferation of blood vessels and connective tissue [42]. This step can go on for the whole life of the implant. During this lifetime, device failure or adverse response can occur (fibrous encapsulation, death of the tissue, clots, etc.). During chronic inflammation, macrophages are the most important cells because they produce a great number of biologically active products, such as growth-promoting factors, cytokines, coagulation factors, etc. The end of the cycle is the healing response, which is initiated by the action of monocytes and macrophages, followed by the proliferation of fibroblasts and vascular endothelial cells. This leads to the formation of granulation tissue at the site of the implant. The proliferation of new blood vessels and fibroblasts is the histological proof for granulation tissue formation and in extension wound healing response. Vince et al. studied the inflammatory reaction after cuff implantation around sciatic nerve in a rat model. They observed that a strong epineural reaction arose after only a few hours. About one day after implantation the nerve is surrounded by inflammatory cells. One week after implantation, surrounding tissue inflammation is replaced by fibrosis and it continues until epineural thickening, consisting mainly of fibroblast and collagen fibers with a reduced number of inflammatory cells [22, 31, 32].

Thil et al. studied the morphological changes following nerve cuff electrodes implantation

along with time, up to one month. By studying parameters linked to the electrodes (impedance, rheobase and chronaxie), they showed the time modification of the interface to stimulation. Their results are summarized in figure 2.9 [22].

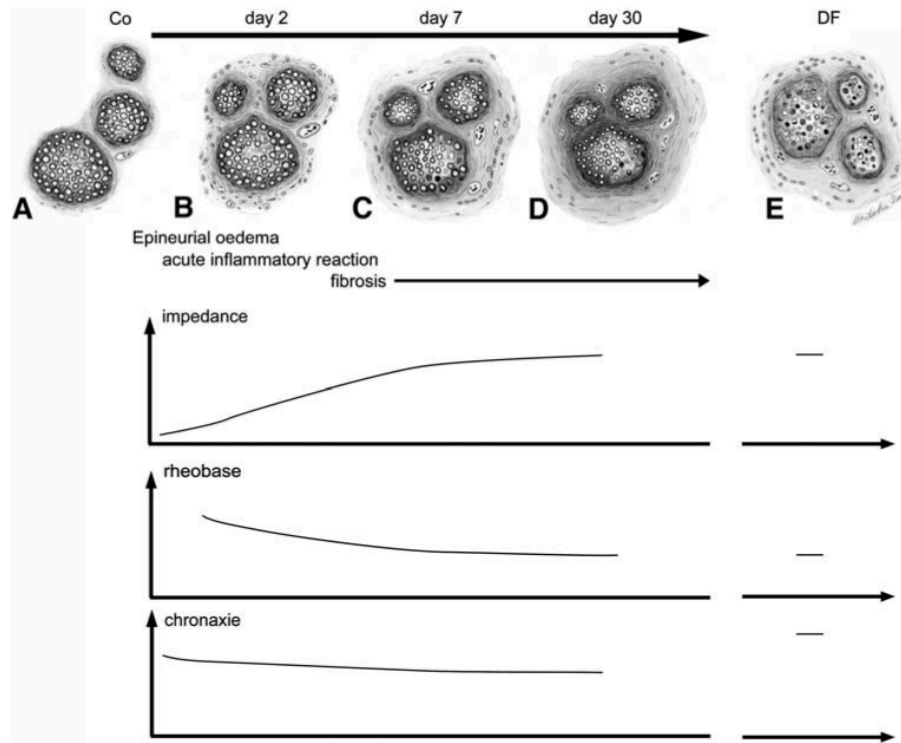


Figure 2.9: Summary of the events taking place in the first month following cuff implantation. Surgical procedure could induce an oedema that may account for the low impedance and high rheobase observed between implantation time and day 2. Compared to control (A), tissues at day 2 (B) were invaded by inflammatory cells and loose fibre deposits. The impedance increased and the rheobase decreased while tissue surrounding the nerve evolved in a dense fibrous tissue (C) that tended to merge with the perineurium at day 30 (D). Only few damaged axonal profiles were observed, localized at the fascicle periphery. No major variation of the chronaxie value was observed. Functional deficit is characterized by axonal degeneration (E) and high chronaxie values. Adapted from Thil et al. [22].

Although they do not reflect the complexity of in vivo situations, the evaluation of biocompatibility can be done using in vitro experiments. By studying the interactions between cells and the material in its use, people can evaluate different parameters : morphological changes, cell adhesion, mediator release, change in metabolism, etc. Though it is a highly simplified model of the biological environment which they have to represent, it is possible by this mean to study certain parameters autonomously. Likewise, people could outcome with new possibilities to create implants with lower inflammatory reaction.

2.2.2.3.B Solutions to reduce inflammatory reaction Nowadays, the reduction of inflammatory reaction after implantation is achieved by the systemic injection of large doses of anti-inflammatory molecules. Systemic injections of dexamethasone, a synthetic glucocorticoid drug with anti-inflammatory effects, have been shown to effectively minimize the reactive tissue response to neural implantation. But this kind of administration is expensive and can cause serious side-effects.

The conclusion of the previous development is that long-term success of a material in the body relies on the controlled macro-functional properties and the physico-chemical properties of the material at different scales [46].

In order to enhance biocompatibility of implanted materials, reduce macrophage adhesion and limit inflammatory reactions, surface modifications are widely studied. It is worth remembering that while in one hand attraction and adhesion is not wanted (macrophages, white blood cells), adhesion of other cells or proteins is sometimes desirable. Surface modification is the key to increase performances of stimulation electrodes. Moreover, one should understand the influences of surface properties on cells grown on them. Lots of studies have been made to distinguish the trends related to different features of surfaces. From what can be seen in literature, we can state that the main material characteristics which influence cell interaction with the surrounding biological medium can be distinguished as: topography, mechanical properties and surface chemistry [46–48].

Moreover, a new emerging solution to reduce inflammatory reactions is the local and controlled delivery of anti-inflammatory molecules by the electrode itself into the direct environment of the implant.

2.3 Conducting Polymers

2.3.1 Definition and general properties

Conducting electroactive polymers (CEP) (or conductive polymers - CP) are a class of materials which possess particular and interesting intrinsic properties. The works of Shirakawa, Louis and MacDiarmid on polyacetylene lead to the discovery, in the mid-1970s [49, 50], of this new class of polymers possessing high electronic conductivity in partially oxidized (or less frequently reduced) state. Since then, conducting polymers have been captivating the interest of researchers because of the numerous opportunities they offer. These researchs also supported the industrial development of conducting polymer products and lead to the fundamental understanding of the physics, chemistry and material science of these materials. They are now used in many applications in a wide spectrum of field ranging from energy storage to implantable biocompatible devices. The principal characteristic of such materials is, by definition, their capacity to conduct electricity. There are different categories of conducting polymers. In this work, only electronically conducting polymers will be discussed (or intrinsically conducting polymers - ICP).

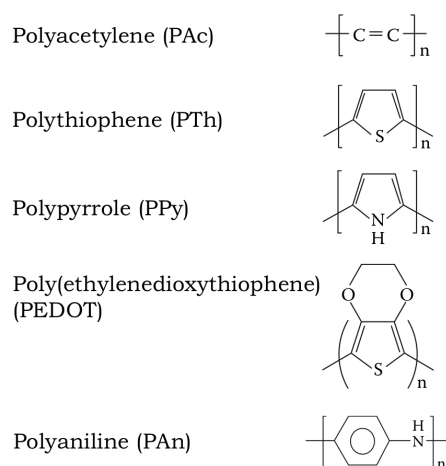


Figure 2.10: Typical conducting Polymer Structures (names and structures) in their undoped form. Adapted from [51]

There are more than 25 reported conducting polymer systems [51] with some of the more commonly studied shown on figure 2.10. Intrinsically conducting polymers all present the same backbone characteristic, *ie* an alternation of double and simple carbon-carbon bonds. It is this particular structure that confers their electronic properties as well as other particular properties. The whole understanding of the fundamental nature of charge propagation has taken 20 years and is concurrent with the understanding of the polymer structure. The transport of electrons in

conducting polymers is assumed to occur via an electron exchange reaction (electron hopping) between neighboring redox sites in redox polymers and by the motion of delocalized electrons through conjugated systems in the case of CP such as polyaniline and polypyrrole [52]. CP have a highly conjugated structure, with the consequence of the overlapping of the sp_2 hybridised orbital. The removal or addition of a π -electron from the conjugated backbone by doping (p-type or n-type, respectively) allows the delocalization of electrons by creating a charge carrier [53]. Electrons can consequently move freely along the polymer chain, allowing the macroscopic conduction of electricity by the polymer. There has been several conduction mechanisms considered (such as variable range electron hopping, fluctuation-induced tunneling [54]). In almost every case, the conduction is not only electronic, but the transport of the charge is also done by the motion of electro-inactive ions during electrolysis (*ie* mixed conductors materials). It is worth noting that owing to the complexity and diversity of these systems, much research is still needed to fully understand all processes (individually or not) related to the properties of conducting polymers and the interacting molecules which are confined therein .

CPs are polyconjugated, polyaromatic or polyheterocyclic macromolecules with a polymer backbone which is in itself electronically conducting in its doped state (contrarily to redox polymers). The doping process of conducting polymers is, as told before, essentially a charge transfer reaction leading to the partial oxidation (or reduction in some cases) of the polymer. In fact, chemically synthesized CP are initially insulators (in a neutral state) and it is only through oxidation (p-doping) or reduction (n-doping) by chemical or electrochemical means that the mobile charge carriers necessary for conductivity are formed. Electronic energy diagrams for polypyrrole can be seen on figure 2.11. The band gap of neutral PPy is reported to be 3.16eV, too wide for electron transfer from the valence to the conduction band at room temperature. The formation of a polaron creates two new intermediate states (bonding and antibonding) within the band gap. The unpaired electron occupies the bonding (low energy) state, thus gives the polaron a spin of 1/2. Further oxidation leads to the removal of another electron from a PPy chain containing a polaron, resulting in the formation of a bipolaron which is energetically preferred to the formation of two polarons. The bipolaron states lie further from the band edges. The lower energy state of the bipolaron is empty; thus the species has a zero spin. As the degree of oxidation increases further, the bipolaronic energy states overlap, resulting in the formation of narrow intermediate band structures.

Most studies of charging mechanisms have been done on p-doped CP (although CP can be charged positively or negatively). Their electrical conductivity is in the range of $1\text{-}1000\text{ S.cm}^{-1}$ in their oxidized (doped) form [55] and are generally insulating in their fully reduced - undoped - state. A maximum conductivity of a CP is reported over 10^5 S.cm^{-1} [56]. The comparison of electrical conductivity of CP with different materials can be found on figure 2.12. As mentioned before, the majority of conducting polymers are conductive due to p-doping, meaning the removal of a π -electron from the polymer backbone leading to a mobile positive charge (electron holes) and creating quasi-particles called solitons, polarons and bi-polarons. These are formed

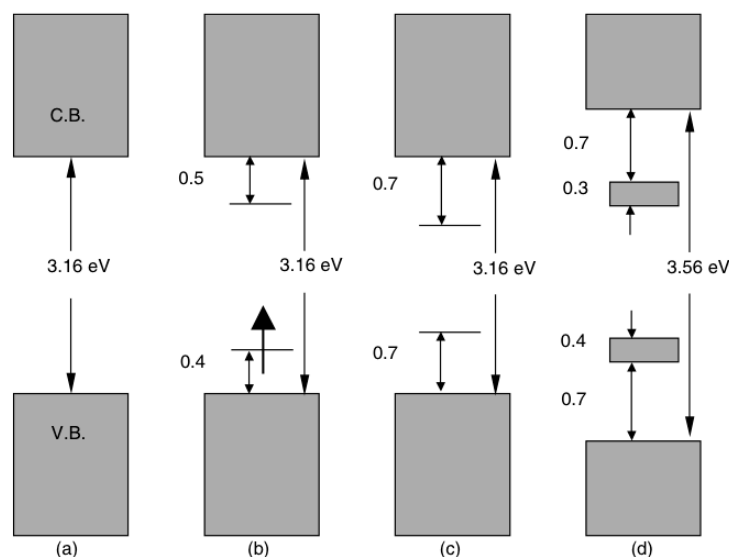


Figure 2.11: Electronic energy diagrams for (a) neutral PPy, (b) polaron, (c) bipolaron, and (d) fully doped PPy. Adapted from Skotheim [55].

when a charge polarizes the surrounding lattice, see figure 2.13.

As a simplification, quasi-particles are presented with a charge localized to one atom, but the quasi-particle is delocalized over several monomer units (typically around 2-4 for a radical cationic polaron on polypyrrole [57, 58]). The charge moves along the chains or less frequently between chains by interchain hopping where the π -orbitals overlap. The interchain conductivity is typically 100 times lower than in the chain direction. Although, this hopping is very important as its absence would lead to complete charge localization. Three-dimensions conductivity depends thus on both chain coherence length and inter-chain hopping probability. It is also important to mention the variation of electrical conductivity with the oxidation state of the polymer. As previously reported and further explained, the polymer can be in different states depending on its oxidation state. As shown on figure 2.14, Felman et al. [59] proved the dependance of conductivity with the potential applied to the film. We can see on this graph that when the polymer is in its reduced state, it is far less conductive than in its oxidized state.

Conducting polymers can be (electro-)chemically switched between their reduced and oxidized states, just by changing the potential or by varying the composition of the contacting fluid media [52]. This behaviour is unique in comparison with the majority of electron-conducting materials (*eg* metals). This change in electrochemical state not only changes the conductivity properties (as mentioned before) but also leads to several modifications in other properties, as shown on table 2.1. These properties will not be explained in detail in this work, but can be found

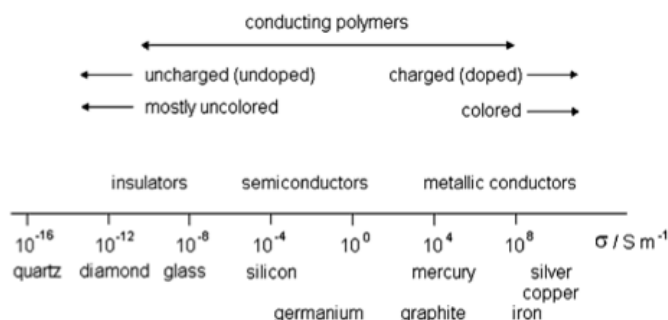


Figure 2.12: Illustration of the range of electronic conductivities of conducting polymers in comparison with those of other materials. Adapted from Inzelt [52]

in [52, 57]. There are numerous applications of conducting polymers linked to the particular

Table 2.1: Quantitative properties of CPs according to their charging state. Adapted from Inzelt [52]

Charging state	Reduced	Oxidized
Stoichiometry	Without anions (or with cations)	With anions (or without cations)
Content of solvent	Small	Higher
Volume	Increase with oxidation	
Colour	Transparent or bright	Dark
Electronic conductivity	Semiconducting	Metallic
Ionic conductivity	Smaller	High
Diffusion of molecules	Dependent on structure	
Surface tension	Hydrophobic	Hydrophilic

properties of these materials. These applications appear in various fields. We can mention some of the most interesting in the context of this work. First of all, CPs were hoped to replace metals in many applications but this has not been realized to date. The development of stable highly conducting polymers with suitable mechanical properties is to be achieved. Although, when requiring relatively low conductivities, a lot of applications have been developed, such as in anti-static or anti-corrosive coatings, microelectronics, ... [60]. Exploiting the conductivity and electroactivity of CPs, an extremely great number of applications have been developed. The charge-discharge property has aroused much interest in the development of rechargeable battery technologies. Recent researches also show the possibility for conducting polymers to be part of supercapacitors and photovoltaic systems [55].

Because of their ability to be doped to a much larger degree than inorganic semiconductors (up to 1 charge per 3 monomer units) and their permeable nature, they offer an enormous charge storage capacity (100 F/g) [61]. The ion flux associated with the redox properties also causes volume changes of the polymer matrix, making these materials suitable for electromechanical actuators used as drug delivery systems or artificial muscles for example. Biomedical applications have also been considered including biosensors, cell growth substrates, implantable

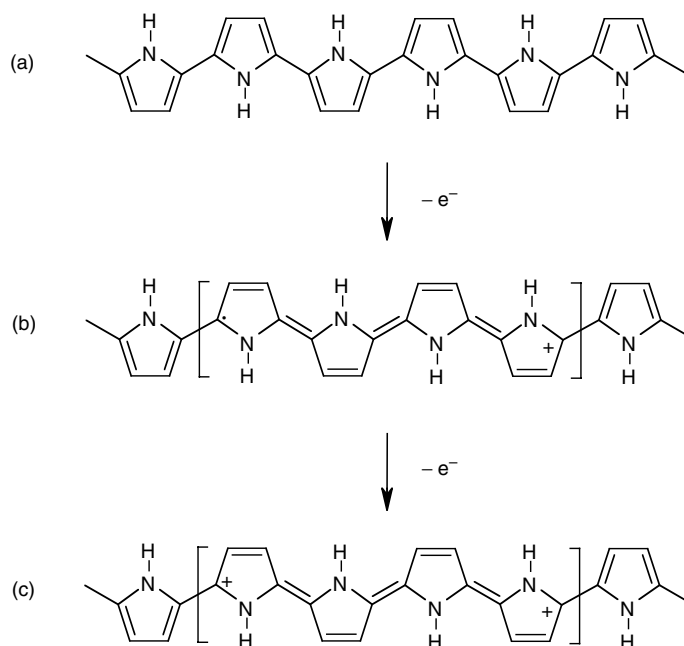


Figure 2.13: Electronic structures of (a) neutral PPy, (b) polaron in partially doped PPy and (c) bipolaron in fully doped PPy. Dots represent unpaired electrons, and + indicates a positive charge. Adapted from Skotheim [51]

electrodes coatings [26, 62]. Considering the incorporation of suitable ionic species during the synthesis of conducting polymer films, they are also ideal hosts for the controlled release of chemical substances including therapeutic drugs, pesticides, fungicides and biologically functional macromolecules (proteins, nucleic acid, ATP, DNA and even whole living cells) among others.

2.3.2 Synthesis of polypyrrole

Amidst conducting polymers, polypyrrole is particularly attractive for the purpose of this thesis and the aims pursued. Polypyrroles (PPy's) are conducting polymers consisting of repetitive units of pyrrole or substituted pyrrole monomer units. The first mention of PPy is reported in the early 1960s in the work of Weiss et al. [63] in which they produced an insoluble, amorphous, black polymer of a 1 S.cm^{-1} conductivity by the pyrolysis of tetraiodopyrrole. They also showed the electrochemical reduction of polypyrrole to remove the iodine dopant [64] and further examined the n- and p-doping effects [65]. But the impact of this work was low. However, since the works of Diaz et al. [66–69], polypyrrole has gained growing interest and is now by far the most studied conducting polymer. The reasons for this particular interest in polypyrrole are multiple. First, the monomer pyrrole is easily oxidized, water soluble and commercially

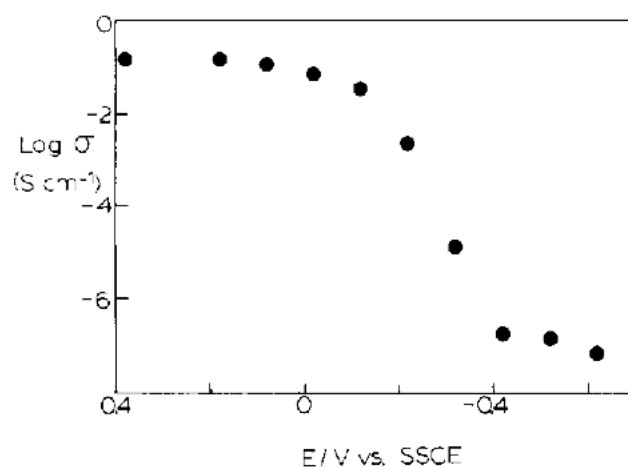


Figure 2.14: Conductivity vs. potential for ClO_4^- doped polypyrrole. Doping induced metal insulator transition is observed at about -0.3V vs SSCE. Adapted from Feldman [59]

available. Hence, polypyrrole presents a lot of advantages such as high electrical conductivity, good environmental stability, good redox properties, biocompatibility, easily synthesized at low potential in an aqueous solution, ... [70] There is a large range of possibilities to tailor the synthesis and properties of polypyrrole, which make this material attractive for a wide range of applications. In this section, the synthesis techniques of polypyrrole will be first discussed, and the possibility to control it.

There are different means to synthesize polypyrroles. But in the vast majority of cases the oxidation of pyrrole monomer units into polypyrrole have been carried out either by electropolymerization at a conductive substrate through the application of an external potential or chemical polymerization in solution by the use of a chemical oxidant [57]. Chemical synthesis is used when large quantities of material are required and the number of experimental variables available to modify the polymer properties is greatly limited. Electrochemical synthesis is extensively used for research purposes due to the simplicity of the technique in the generation of good quality films and the control over a large range of the polymer properties as well as the wide choice of dopant ions [60]. In this work, only electrochemical techniques will be used to synthesize polypyrrole films. The electrosynthesis mechanism will thus be explained in detail in this section. As intrinsic properties of polypyrrole (structure, conductivity, mechanical and chemical properties, etc.) are highly dependent on electropolymerization conditions, the influence of the technique and parameters, as well as electrolyte composition (type, concentration, pH) and temperature will also be explained.

2.3.2.1 Mechanism

The electrochemical polymerization consists in the oxidation at a surface of an electrode of a monomer by the application of an anodic potential. This monomer is dissolved in an appropriate solvent containing the selected anionic doping salt. Its choice is of particular importance as both solvent and electrolyte has to be stable at the oxidation potential of the monomer and provide an ionically conductive medium. Since pyrrole has a relatively low oxidation potential [71] electropolymerization can be carried out in aqueous electrolytes. Organic solvents like acetonitrile or propylene carbonate are also commonly used for their high relative permittivities and wide potential windows [72]. The general overall electropolymerisation of polypyrrole consists in the chain oxidation of a pyrrole monomer into long chains of polypyrrole (see figure 2.15). During this electrochemical synthesis, charges are formed onto the polypyrrole backbone and ions (A^-) (called in this case *counter-ions*) are incorporated inside the bulk of the film, in order to ensure the electrostatic charge balance. The level of oxidation (represented by n) is the number of monomer units for every charge created, and depends on several synthesis parameters. The resulting polymer is thus produced in the oxidized state with incorporation of counter-ions. The counter-ion content is usually high and incorporated between the PPy planes [73]. These plans are predominantly $\alpha - \alpha$ bonded [74] forming long polymer chains. In reality, the molecular structure of PPy is different because of the highly insoluble and highly crosslinked materials formed. Behind this simple electropolymerization process leading to an ideal structure of polymer is hidden the complexity and the dynamic character of conducting polymers. In fact, the electropolymerization mechanism of polypyrrole is not yet fully understood and a certain number of mechanisms have been proposed to date. This is principally because of the rapidity of the polymerization coupled with the insoluble and non-crystalline natures of polypyrrole, which make further analysis exceedingly difficult. In this work, the mechanism of Diaz will be presented as the most often encountered in the literature [70, 75].

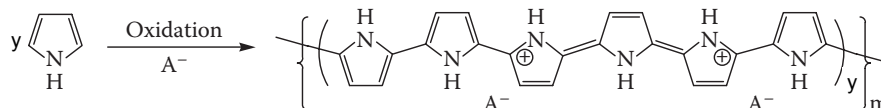


Figure 2.15: Polypyrrole : general synthesis reaction and structure. Adapted from Wallace et al. [57]

The polymerization begins by electron transfer (E) followed by a succession of chemical reactions (C) and electron transfer reactions. So, the reactions involved in the formation of the polymer can be summarized as $E(CE)_n$, as commonly seen [76]. The polymerization of polypyrrole can be considered as a step polymerization process, as described here.

- **Step 1:** The oxidation of a monomer R at the surface of the electrode to form a radical cation $R^{+\bullet}$, with different resonance forms. Because the electron transfer reaction is faster

than the diffusion of R in the solution at the electrode interface, this leads to the high concentration of $R^{+\bullet}$ in the surroundings of the electrode. Considering their reactivity, these monomeric radical cations can undergo different reactions :

- Relatively stable $R^{+\bullet}$ can diffuse into the solution where they react to form soluble products with low molecular weight ;
- Very unstable $R^{+\bullet}$ can react rapidly near the electrode with the solvent or anions to form soluble products of low molecular weight ;
- Between those two situations, $R^{+\bullet}$ can also undergo dimerization reactions, leading to step 2.

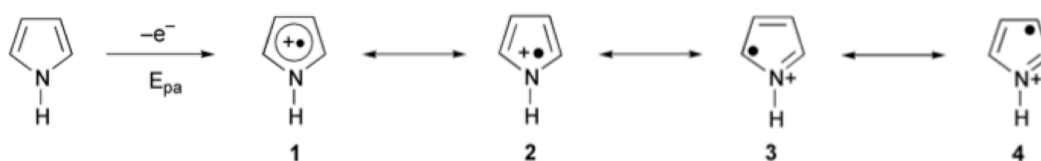


Figure 2.16: Diaz mechanism : Step 1. Adapted from Sadki et al. [70].

- **Step 2:** The coupling of two radical cations $R^{+\bullet}$ resulting in the formation of a dihydromer dication. The $R^{+\bullet}$ involved have a greater unpaired electron density in the α -position and dimerize via a bond between them.
- **Step 3:** This stabilization step leads to the formation of an aromatic dimer by the loss of two protons.

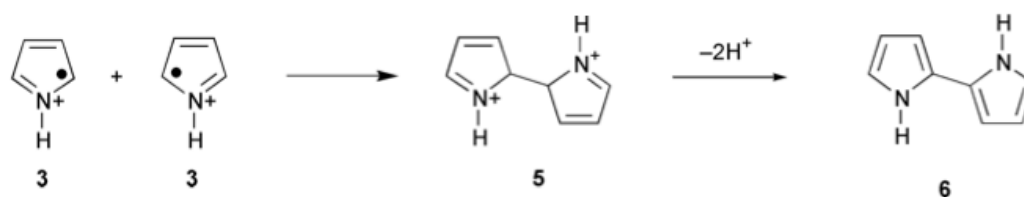


Figure 2.17: Diaz mechanism : Steps 2 and 3. Adapted from Sadki et al. [70].

- **Step 4:** The reaction of the dimer into a radical cation results in the delocalization of the unpaired electron on two rings, with the corresponding resonance forms. The oxidation potential of a dimer is thus lower than the one of a monomer. Therefore, a dimer is more easily oxidized at the applied potential (*ie* oxidation potential of the monomer). This also means that the as-created dimer radical cation is less reactive than the monomer. The resonance form 9 is predominant.

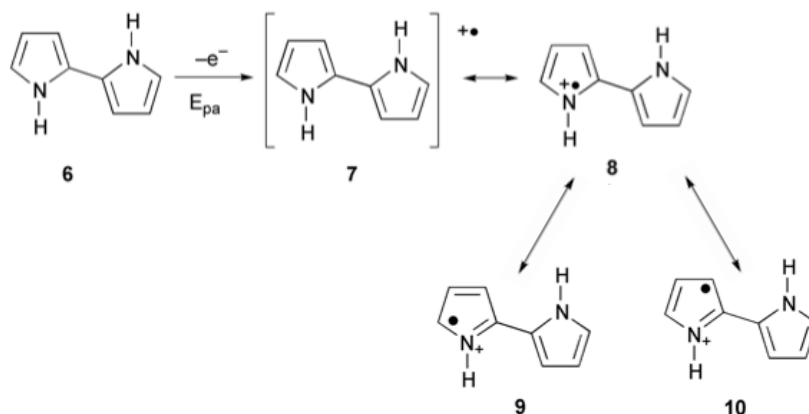


Figure 2.18: Diaz mechanism : Step 4. Adapted from Sadki et al. [70].

- **Step 5:** The resonance form **9** reacts by its 5 or 5' position with a radical cation $R^{+\bullet}$ to form a trimer dication and then a neutral trimer after deprotonation.

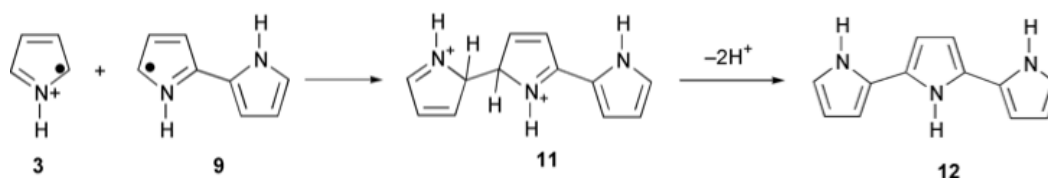


Figure 2.19: Diaz mechanism : Step 5. Adapted from Sadki et al. [70].

- **Step 6:** The electro-oxidation of the trimer leads to the radical cation **14**, with its corresponding resonance forms. At this point, it is worth noting that α -coupling will predominate. However, as the length of the oligomer, the delocalization of the unpaired electron increases, and α -coupling is no longer the only coupling. In fact, the longer the chain, the higher the number of β -bondings. These chain ruptures are responsible for the poor crystallinity of PPy [77].
- **Step 7:** The propagation sequence continues (oxidation, coupling, deprotonation) and the polymer chain grows until the requirements for polymerization are no longer respected.

The final polymer structure is represented on figure 2.21. The structure of the as synthesized polypyrrole is not the neutral (undoped, left) non-conducting form, but the oxidized conducting (doped, right) form. The final polymer chain carries a positive charge every sequence of a few monomer units (between 3 and 4 in general, depending on different parameters).

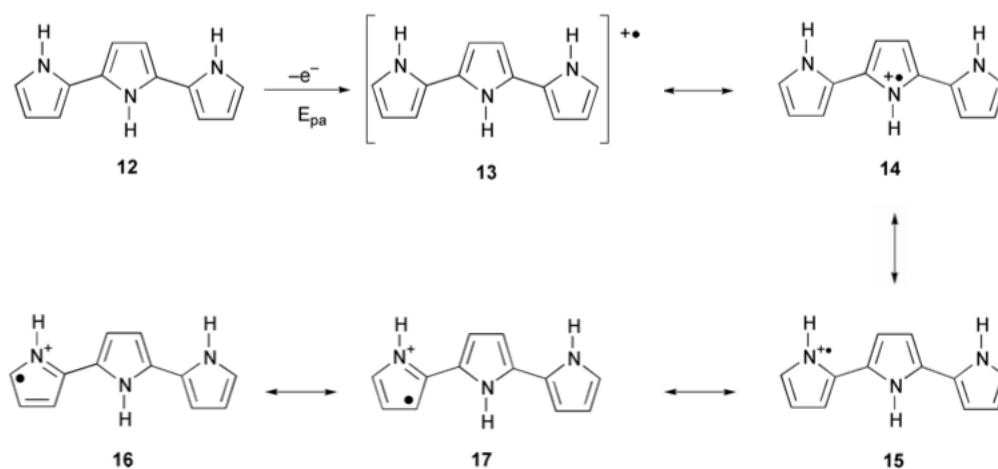


Figure 2.20: Diaz mechanism : Step 6. Adapted from Sadki et al. [70].

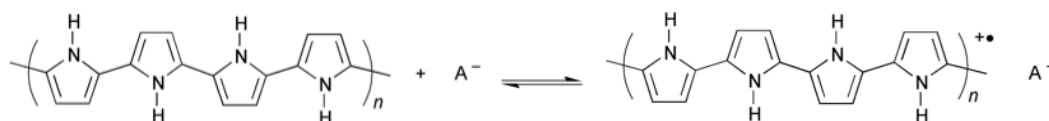


Figure 2.21: Polypyrrole backbone oxidation and reduction. Adapted from Sadki et al. [70].

The global reaction of polypyrrole electropolymerization can be summarized as on figure 2.22. From this mechanism, we can see that $2n$ electrons are required for the film formation. The value determined from the electrical charge for the electropolymerization process is however generally found between 2 and $2.7n$, the excess charge being consumed by polymer oxidation. The determination of this value can be done via the elementary analysis of the polymer, as it is in agreement with the proportion of anions present in the polymer [78].

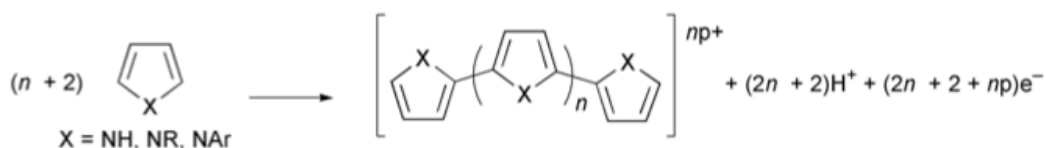


Figure 2.22: Global polymerization reaction of polypyrrole. Adapted from Sadki et al. [70].

In summary, the result of initial oxidation is a radical cation reacting with other monomers present in the solution to form oligomeric products and then the polymer. The conjugation of the growing chain results in the lowering of the oxidation potential compared to the monomer. This is the reason why the synthesis and doping are done simultaneously. An anion is inserted in the polymer during synthesis to ensure the electro-neutrality of the polymer backbone. Even though the Diaz mechanism is the most commonly accepted, controversy still exists on the actual steps of polypyrrole formation.

The first step of monomer oxidation has been proved to be slow [79], while the following steps (radical-radical coupling, deprotonation and subsequent oxidation) are fast. The polymerization is believed to proceed via a radical-radical coupling mechanism [80] in which the repulsions of radicals are prevented by the solvent, electrolyte and monomer. The growth of the polymer is done in solution as the chain length is relatively short, and is believed to finally precipitate on the electrode surface as the solution limit is exceeded. At the end of the synthesis, a polymeric film is formed at the surface of the anode, with its characteristics depending on the following parameters: substrate, solvent, monomer, electrolyte (dopant), preparation conditions, pH and T° (among others).

2.3.2.2 Parameters influencing PPy synthesis

2.3.2.2.A Effect of the substrate or electrode material The substrate of the electrode on which the synthesis is done is of interest especially in the initial steps of growth. As a matter of fact, the properties of the substrate influence the oxidation of pyrrole monomers, as well as the adsorption of the polymer during electrosynthesis. The surface energy of the electrode also determines the occurrence of the deposition and influences the adhesion of the deposited polymer onto the substrate. The nature of the substrate determines the character of the bond between monomer and anode [81, 82]. Deposition of polypyrrole on substrates undergoing non-conductive metal-oxide layer formation (Ti, Fe, Al) has been proved to be difficult, as a consequence of its impediment to the electron transfer during electropolymerization [83]. The microstructure and the size of the working electrode are also of importance on the properties of the synthesized polypyrrole.

2.3.2.2.B Effect of the solvent The solvent is more than just a reaction medium, as its nature will determine the solubility of the resultant polymer. Its choice must consider the nucleophilic reactions that can occur during polymerization, leading to the ending of the propagation step. Aprotic solvents thus appear to be the best choice (ex. acetonitrile, the most commonly used). Nucleophilic solvents do not allow polymer formation (ex: dimethylformamide) unless a protic acid is added to the solution (ex: p-toluenesulfonic acid) [70, 84]. Similarly, high salt concentrations are needed when using aqueous solution to obtain a polymer with the appropriate mechanical and conducting properties. The presence of water in a different solvent (acetonitrile) has been widely studied, as it strongly influences the kinetics of the reaction and the properties of the polymer formed. The solvent influence can be explained by the strength of its interactions with the radical cations and by its polarity [85].

2.3.2.2.C Effect of the monomer The monomer unit constituting the backbone of the polymer chain can be substituted with chemical groups to obtain polymers with peculiar properties.

This substitution and the properties of these groups have an influence on the electrochemical synthesis. As a general remark, the possibility of coupling in β -positions of pyrrole is a disadvantage. Indeed, β -couplings leads to conjugation ruptures and induce a decrease in conductivity and an increase of the band gap. As a proof, the synthesis of α -disubstituted monomer produces only soluble species and α -monosubstituted monomers yield to low molecular weight polymer [86]. So β -substituted pyrroles are preferred, as the β -bonding is prevented and the cristallinity of the polymer is increased. On the other hand, steric effects of this substitution have to be considered as well as the influence of positions 3- or 4- substituents on the electron density of the pyrrole ring [71]. The oxidation potential of such modified pyrrole monomers are thus influenced by the presence of substitution groups on the pyrrole ring.

An important number of N-substituted pyrroles have also been prepared. Such synthesized polypyrroles exhibit lower electrical conductivities up to three orders of magnitude [69]. It is explained by the steric effect induced by the substituent on the nitrogen atom, with a reduction of the conductivity proportional to its size. Waltman et al. [87] examined the effects of size increasing N-substituted alkyl groups on polymerization. They showed that large substituents on the nitrogen atom, or even the β -carbon, induce a stabilization of the radical cation (without interruption of the polymerization process) which can diffuse into the bulk of the solution and form soluble products therein. The yield and molecular weight of the produced polypyrroles are then low. In addition, some specific substituents can induce a phenomenon of self-doping. It means that the monomers can themselves provide the charge needed to guaranty electroneutrality during electropolymerization. The counter-ion content is in this case lowered. As seen on the electropolymerization process, the oxidation of monomer units into polymer is continuous as the film forms at the electrode surface.

2.3.2.2.D Effect of the electrolyte Nature and concentration of the species present in the monomeric solution both influence the solution conductivity, polymerization process and properties of the finally produced polypyrrole. The effect of dopant concentration reveals certain trends, even if all impacts are not yet understood. In general and in a certain range, an elevated concentration of dopant molecules lead to high conductive polypyrrole films [88]. Moreover, the concentration of counter-ions not only has an effect on the amount of anion incorporated in the film but also on the structure and morphology [89]. The choice of dopant is guided by some considerations about their properties. First, the oxidation potential of the doping molecules have to be higher than the monomer in order to avoid the interference of breakdown products in the process. Moreover, organic or inorganic, the dopant molecules can be categorized by their size, which determine (at least partially) the porosity and microstructure of the final polymer and the diffusion of dopants during the redox process [90]. Nevertheless, the synthesis of polypyrrole with large aromatic sulfonate anions (in particular, para-toluene sulfonate) has been proved particularly effective to create films with good conductivity and mechanical properties. This

improvement is due to the degree of cristallinity induced by benzene sulfonates [91], giving a higher conductivity and enabling the polymerization process to occur efficiently. The surfactant nature of sulfonated aromatics is also suggested to play a role by the protection from unwanted side reactions with oxygen, solvent or other nucleophiles.

2.3.2.2.E Effect of pH The monomer oxidation has been proved independent of the pH of the solution, but even though, the reactivity and stability of the polypyrrole formed is pH-dependent [92]. As seen on the general polymerization mechanism, protons are produced after each oxidation step. The pH in the surrounding of the electrode thus decreases. Modulating the pH is also important to insure the creation of a uniform film with good properties. The optimal pH is dependent of the species present in the electrolyte. A low pH will generally enhance polymerization. Under a certain value, it will be responsible for the weak conductivity, because of side reactions creating non-conjugated oligomers that will react with the conjugated PPy while incorporated into the film or directly in the solution [93]. Synthesized polypyrrole stability in aqueous solution is also pH sensitive. In fact, a protonation (deprotonation) process is observed in strong acidic (basic) solutions modifying consequently the conductivity, as mentioned before.

2.3.2.2.F Effect of the temperature On electropolymerization, temperature substantially influences the kinetics of synthesis, and mechanical and redox properties [94]. It is a general trend to observe higher conductivities for lower synthesis temperature. Structures at low temperatures are shown to be much more regular, due to the absence of side-reactions (solvent discharge, nucleophilic attacks on polymeric radicals) creating structural defects [78].

2.3.2.2.G Effect of the electrochemical method and parameters It has been pointed out by Otero et al. [95] that the choice of the electrochemical method can drastically influence the morphology, appearance and adhesion of the polypyrrole. This phenomenon was later reported in numerous works, confirming that the choice of electrochemical conditions and parameters is an easy way to improve the properties of polypyrrole. The simplest way to induce polypyrrole formation is the application of either a constant potential or current. In the case of constant potential polymerization (so-called chronoamperometric synthesis) the choice of the potential determines the rate of oxidation of the monomer and thus the rate of polymerization. As a consequence, if this potential is too low (polymerization too slow), the oxidation of the monomer is slowed down. The critical chain length inducing deposition on the electrode may not be reached before the products leave the reaction zone, leading to soluble oligomeric products. Increasing the potential lead to an increase of the polymerization rate. Nevertheless, the upper value of the potential to be applied is regulated by the side reactions that can occur. This results in the overoxidation of the polymer, leading to the ruptures in the aromatic cycles on oxygen grafting [96, 97]. The result is a porous polymer with poor mechanical and electrical

properties.

Another consideration linked with the nature of the electrode is that different electrodes lead to different coatings. Moreover, the chronoamperometric deposit is sensitive to current drop effects, linked to the electrode resistance (R) increase during synthesis. The actual potential applied on the surface in contact with electrolyte drops and it changes the growth characteristics [55]. However, a lot of works use the chronoamperometric synthesis because it allows a permanent control of the upper potential applied, avoiding the previously described overoxidation. In the case of constant current polymerization (so-called galvanostatic or chronopotentiometric synthesis) the rate of polymerization is dictated by the current density applied. This allows to avoid the potential drop effect leading to more evenly grown polymer films although local variations of current density cause heterogeneous structures. In the same way, if the rate is too low, oxidation without deposition arise; if it is too high, overoxidation occurs. The latter is the main restriction on the use of chronopotentiometric techniques for the synthesis of polypyrrole electroactive films, because of the non-permanent control on applied potential.

Polypyrrole films produced with these techniques are reported to be dendritic and poorly adhesive [98]. Transient potential/current waveforms methods are widely used to overcome the constant techniques limitations. The films obtained by these techniques are reported very adhesive, smooth and homogeneous. Cyclic voltammetry is the most commonly used of these techniques, such as in the work of Mitchell et al. [99]. The films produced were more ordered and anisotropic, due according to them to a larger number of nucleation sites in the growth process [70]. As the potential/current sweeps during synthesis, the redox properties of the already synthesized polymer must be considered in the further polymerization process. Many other techniques using transient waveforms are now used to create more dense, adherent, electroactive, etc; films. Among the most used, we can mention the pulsed current and potential synthesis.

2.3.2.3 Drug delivery properties

2.3.2.3.A General principle As explained previously, the particularly interesting property of PPy in the scope of this thesis is its ability to remove or add charges into the polymer by switching the material between its oxidized or neutral states. This phenomenon is often referred to as the redox properties of the polypyrrole. This switching between redox states is accompanied by charge transfers in or out of the polymer, as it can be seen on figure 2.23.

We can see on this scheme that several parameters are involved in the general redox cycling, leading to different situations. Basically, we can at this point distinguish two main paths for ion exchange between the polymer and the electrolyte in contact. After electrochemical synthesis, the polymer is composed of a charged polypyrrole backbone with counter-anions to guaranty the electroneutrality, as presented before. Some solvent molecules are also present in the film. The representation of the beginning situation is the case B of the figure 2.23. On the first situation

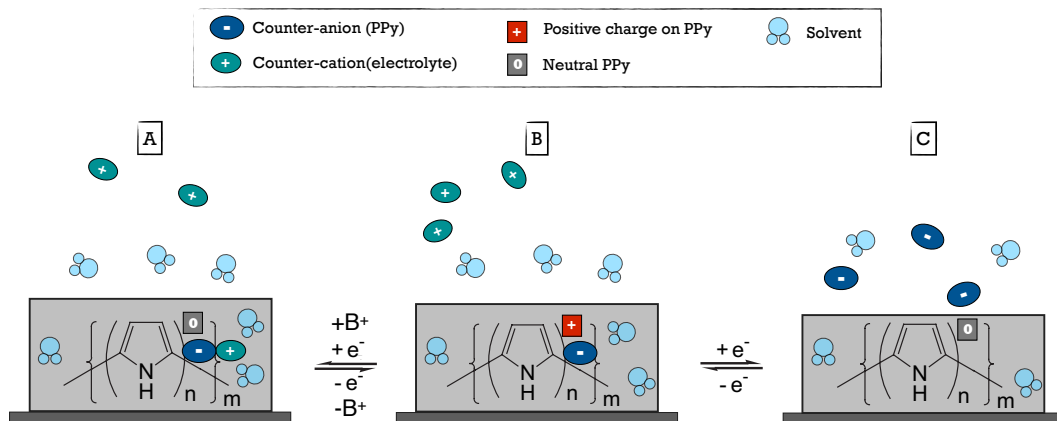


Figure 2.23: Electrochemical behaviour of PPy doped with motile anion in contact with an electrolyte.

(anions exchange path), starting from case B on the scheme, anions A^- are expelled (*ie* they diffuse) into the electrolyte during the reduction process ($+e^-$ direction) to obtain the case C. There is also a movement of solvent molecules from the film. The polypyrrole backbone is thus neutral and the dopant ions can be found in the electrolyte. Upon (re-)oxidation ($-e^-$ direction), the polypyrrole backbone is ionized and dopant anions are incorporated into the film to ensure electroneutrality. These ions can be either the previously ejected anions or other anions present in the electrolyte medium

In the second situation, anions A^- are immobilized counter-ions. Starting from case B, the reduction mechanism involves neutralization of the polymer backbone. The negative charges of immobile counter-ions that are not anymore counterbalanced by the polymer backbone and therefore the entry of cations B^+ from the electrolyte medium is observed, as represented on case A. The inverse phenomenon is observed upon (re-)oxidation. Solvent movement is also to take into account. A lot of studies have been done to determinate and illustrate these affirmations [100–102]. These reactions are accompanied by mechanical -or structural- changes in the film, as a consequence of ions and solvent movements from and into the film. The parameters ruling the occurrence of one or the other situation will be discussed in details in the following sections, as well as the parameters linked to their progression. Chemically, the situations can be transcribed by the following chemical reactions (see 2.24) [100, 103].

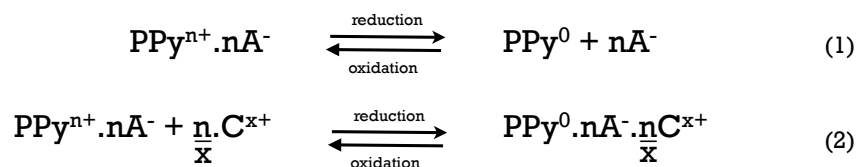


Figure 2.24: Redox reactions paths for polypyrrole.

where A^- is a counter-ion present in the film after synthesis and C^{x+} a cation present in the

electrolyte. Equation (1) corresponds to the first path and equation (2) to the second. As a general statement, we can mention at this moment that the movement of ions in/out of the film is accompanied by solvent movement. This will also be explained, as an important deviation from generally simplified interpretations of redox properties of polypyrrole.

Over the years, a lot of work has been done by researchers to study the release of molecules by polypyrrole and the means to control it. It has been shown that the polymer goes into several modifications during redox switching, affecting its properties and the further comportment for liberation. Although a lot of efforts have been made to clearly understand the mechanisms behind the drug delivery by polypyrrole, many shadow zones still remain. Yet, some general trends can be stand out. In this work, we will focus more on a limited number of them, namely the counter-ions (size, charge, structure), film parameters (thickness, density, microstructure) and the electrochemical liberation methods (technique and parameters). In a first time, the next section is a summary of the previous work about drug delivery by polypyrrole in order to settle the scope of this work.

On figures 2.25 and 2.26, we can see a list of some of the most interesting works about drug delivery by polypyrrole, with the corresponding synthesis techniques and parameters, the molecules to be released, the liberation techniques and parameters, the release medium and the analysis carried out.

A rapid look at the synthesis techniques confirms the previously explained influences on film properties. The nature of drug/charge released shows the wide variety of molecules that can be liberated from polypyrrole. Biomolecules (or biologically active molecules) are the major part of them. We can distinguish them also by their electronic nature as their process for release in of opposite nature (*cfr* previous considerations). Stimulation techniques can be separated into three : cyclic voltammetry, potential steps and current pulses. This is justified by the needs on one hand for research on liberation process and on the other hand by in vivo limitations. Measurements and analysis enlighten the most used techniques to study drug delivery by polypyrrole, as an introduction to what will be used in this thesis. Based on these works and other works, general trends have been outlined and linked with the parameters applied. The next section is a general overview of them.

Authors	Materials and synthesis	Drug/charge released	Stimulation technique / parameters	Electrolyte	Analysis	Ref
Zinger et al (1984)	PPy/Cl ⁻ ; "anodic current" deposition, 5WV (-1V then 0V)	Glutamate (anion)	Zn ²⁺ cathodic pulse (-1V) : 20 pulses	-	Amino-acid analysis	[127]
Li et al (1992)	Py(-) / pTS (-); E=0.8V followed by 5WV (-0.6 ; 0.6V) ATP	adenosine 5' - triphosphate (anion)	Potential steps (-0.6 - 0.6V)	NaCl	UV abs	[135]
Creed et al (1994)	Py(-) / acetyl/salicylate (-) - salicylate (?), E = 1V vs SCE	acetyl/salicylate (anion), salicylate (anion)	reduction at -1V & CV	-	spectrophotometry of Fe(III) salicylate IR bands	[136]
Hegel et al (1994)	Py(-) / melamin (-); electropolymerization	melamin	cyclic voltammetry (unclear)	-	EQCM	[137]
Pyo et al (1994)	PPy / ATP ; E = 0.8V	ATP (anion)	CV (-1 - 0V, mV/s)	-	EQCM, UV abs	[138]
Lin et al (1994)	Py(0.25M) / AQSA(0.025M) ; I=0.2mA/cm ²	AQSA (anion)	Potential steps (-0.8V)	-	-	[118]
Gandhi et al (1995)	Py(0.2M) / HpTS(0.05M) (I=3mA/cm ² , T=10-13°C, pH3) / PVS (-)	pTS (anion) - PVS (anion)	Potential steps (-0.8V), CV (-0.8 - 0.4V, 10mV/s)	XNO3(1M), NaPVS(1M)	tensile test	[139]
Li et al (1995)	Py(-) / pTS (-); anodic polymerization on Pt electrode followed by anodic oxidation of films in an ATP solution	ATP (anion)	reduction	aqueous electrolyte solution	UV abs	[88]
Hegel et al (1996)	Py(0.2-0.7M) / NaCl(0.1M) / A (0.002-0.01M) ; Potential step	hydroquinone - catechol - anthraquinone2sulfonate - melamin	CV (-1V - 0.4V, 50mV/s)	various aqueous solutions	EQCM	[101]
Pyo et al (1996)	Py(-) / ATP (-); E=0.8V vs Ag/AgCl	ATP (anion)	cyclic voltammetry	-	UV abs	[140]
Hegel et al (1997)	PPy/Cl ⁻ & PPy/melamin ; E=0.6V	Chlorpromazin	Potential steps (-800 - +200/400mV)	-	UV abs, EQCM	[102]
Kontturi et al (1998)	Py(0.1M) / A (0.1M) ; E = 1V	A = Tosylate, Salicylate, Nicotinic, Naproxen (anion)	Potential steps (-300 -500, -800mV)	0.1M monomer-free 0.02M ATP, 0.1M NaCl / Na2HPO4	EQCM, HPLC	[106]
Pernaut et al (2000)	Py(0.25M) / ATP(20mM) ; E=0.5-1V / I=0.1-10mA/cm ²	ATP (anion)	Potential steps (-0.4 - -0.8V)	-	UV abs, EQCM	[132]
Collier et al (2000)	Py(0.1M) / HA(2mg/ml) / PSS(0-0.1M sulfonate) ; E=720mV	hyaluronic acid (anion), pTS (anion)	CV (-0.8 - 0.4V, 10mV/s)	NaCl(0.1M)	SEM, XPS, RAIR, BHAF, in vivo	[141]
Massoumi et al (2001)	Bilayers : Py(0.1M) / SSA (0.1M, E=0.85V vs SCE ; NMPPy(0.1M) / PSS (0.1M), E=0.95V vs SCE	sulfosalicylic acid (SSA) (anion)	CV (-0.6 - -0.7V, 50mV/s), cathodic potential <-0.5V	0.1M LiClO4 in H2O	UV abs	[142]
Massoumi et al (2002)	Bilayers : Py(0.1M) / DMPPA(20mM), E=0.85V vs SCE ; PMMP(0.1M) / PSS(0.1M), E=0.9V vs SCE	dexamethasone phosphoric acid (DMPPA) (anion)	reduction at <-0.6V	0.1M LiClO4 in H2O	EQCM, UV abs	[128]
Grzeszczuk et al (2004)	Py(0.6M) / Na2ATF6 (0.04M), E=0.8V	ATF6 3- (anion)	CV (-1.2V - 0.6V, 0.001 - 0.2V/s)	Na2ATF6(0.04M, pH4), NaCl(0.1M, pH3), pro(0.05M)	EQCM	[143]
Li et al (2005)	PPy/Cl ⁻ ; E=8V (-) + Poly(vinyl-alcohol) (PVA) -heparin hydrogel	heparin	Current steps using PPy as wire (0.1, 3.5mA ; 120min-24h)	-	heparin assay	[144]
Wadhwa et al (2006)	Py(0.1M) / DEX (0.1M) ; E=1.8V	DEX (anion)	CV (-0.8 - 1.4V, 100mV/s)	PBS(0.1M, pH 7.2)	UV abs, FTIR, cell culture	[103]

Figure 2.25: Literature summary of materials, synthesis and release conditions for PPy based delivery systems (1/2)

Authors	Materials and synthesis	Drug/charge released	Stimulation technique / parameters	Electrolyte	Analysis	Ref
Tompson et al (2006)	PY(0.2M) / PTSG(0.05M) / NT3 (0-24g/ml) ; $I=2mA/cm^2$ for 90s-40min	NT3 (neutral)	CV (-0.8 - 1V, 50mV/s) ; Potential pulses (-0.6 - 0.6V, 5Hz) ; Current pulses (+/-0.5 ; +/- 0.20mA)	0.9% NaCl	SEM, Gamma	[26]
Georges et al (2006)	PY(0.1M) / SDS(0.02M) / boatin (0.0082M) ; $2mA/cm^2$	NGF (neutral)	Potential steps (-3V)	-	-	[6]
Ocalp et al (2006)	PY(-) / hexacyanoferrate(-)	hexacyanoferrate (anion)	Constant potential at -0.4V and <-0.6V	-	-	[145]
Li et al (2007)	PY(0.1M) / ATP(0.02M) ; E=0.6V	ATP (anion)	CV (-1.1 - 0V, 10mV/s) ; potential steps (0V, 1min + 1.1V, 1min)	-	-	[126]
Dong-Hwan et al (2007)	PY(0.2M) / PSS(0.1M) / KGF (100g), $I=0.5mA/cm^2$	NGF (neutral)	CV (-0.9 - 0.5V, 100mV/s)	PBS(0.1M, pH7)	EIS, SEM, cell culture	[146]
Abizem et al (2007)	PY(0.1M) / NaHS(0.1M) / PY(0.7M) / NaNO ₃ (0.07M), E=0.8-0.9V vs SCE	toluene sulfonate (anion), salicylate (anion), naproxen (anion)	Constant potentials from -0.4V to -0.7V vs SCE	monomer free solution	EQCM, UV abs	[147]
Richardson et al (2007)	PY(0.2M) / PTSG(0.05M) / NT3(0-24g/ml), $I=2mA/cm^2$ for 90s-60min	NT3 (neutral)	Charged-balanced biphasic current pulses (250Hz, +/-1mA/cm ² , 100µs width, 20µs interpulse, 3.78ms short-circuit) for 1h	1 ml DMEM/Sito	SEM, cell culture, radio labelled	[148]
Xiao et al (2007)	PY(0.1M) / TSN(0.05 - 0.2M), E=0.6V, 5-60min ; CV (-0.1V - 0.6V) then E=-0.6V in NaCl(0.1M) ; E=0.6V in ATP solution	ATP (anion)	Constant potential at -0.6V and CV (-1V - 1V)	0.1M NaCl	SEM, EIS, UV abs	[149]
Richardson et al (2009)	PY(0.2M) / PTSG(0.05M) / NT3 (0-24g/ml) ; $I=2mA/cm^2$ for 90s-40min	NT3 (neutral)	Current pulses (100µs, 250Hz, 350-825µA) 8h/day	in-vivo	EIS, in-vivo	[150]
Luo et al (2009)	PY(0.2M) / Ru(0.01M) (anion or flu) + PY(0.2M) / Ru(0.01M) or DEX(0.02M) (C/CV 0.5 - 1.2V), 50mV/s)	Flu (anion) - Ru + DEX (anion)	Potential steps (-0.5 or -1.2V, 5s + 0V, 5s) 14day for 3days	PBS(0.01M)	SEM, UV abs	[151]
Gee et al (2009)	PY(0.2M) / SS(0.2M), $I=2mA/cm^2$, 100-500s or ATP(0.05M), E=0.85V Q=1mC	SSA (anion), ATP (anion)	Potential steps (-0.5, -0.8, -1V)	PBS(0.1M, pH7.2)	UV abs, cell culture	[152]
Srivastav et al (2009)	PY(0.1M) / P/SG (82mM) or DEX(0.125mM) / NaCl(1M, pH7.2), CV(0 - 1.1V, 100mV/s, SCV)	pendiliv/streptomycin (P/S) (anion), DEX (anion)	CV (-1V - 1V, 10mV/s)	PBS(pH7.2)	AFM, XPS, cell culture, UV abs	[153]
Evans et al (2009)	PY(0.2M) / PTSG(0.05M) / BDNF(0-24g/ml) ; $I=2mA/cm^2$ for 90s-60min	BDNF (neutral)	Charged-balanced biphasic current pulses (250Hz, +/-0.2mA, 100µs width, 25µs interpulse, 3.78ms short-circuit - 0.1µC/cm ²) for 4 days (8h stimulation, 16h rest)	0.9% NaCl	SEM, ELISA, cell culture	[154]
Syvakis et al (2010)	PY(0.2M) / PTSG(0.1M) / resveratrol (0.0.05M), $I=2mA/cm^2$ for 90s ; $2I=2mA/cm^2$ for 90s	resveratrol (neutral)	Potential steps (+/-0.6V, 120s + potential pulses (+/-0.6V, 5Hz, 380s)	ACN:Me ₂ PO ₄ (45:55, v/v)	HRIC, SEM, AFM	[155]
Tompson et al (2010)	PY(0.2M) / PTSG(0.05M) / NT3+BDNF(0.24g/ml), $I=2mA/cm^2$ for 90s-60min	NT3, BDNF (neutral)	Charged-balanced biphasic current pulses (250Hz, +/-0.2mA, 100µs width, 25µs interpulse, 3.78ms short-circuit - 0.1µC/cm ²) for 4 days (8h stimulation, 16h rest)	0.9% NaCl	SEM, cell culture, radio labelled, ELISA	[156]
Tompson et al (2011)	PY(0.2M) / PTSG(0.05M) / NT3(0.24g/ml), $I=2mA/cm^2$ for 90s-60min ; PY(0.2M) / PMS-MA-CG(0.05M) / NT3(0-24g/ml), $I=0.2mA/cm^2$ for 12min-8h	NT3 (neutral)	Charged-balanced biphasic current pulses (250Hz, +/-1mA/cm ² , 100µs width, 20µs interpulse, 3.78ms short-circuit) for 1h	1 ml DMEM/Sito	SEM, CV, Contact angle, radio labelled, cell culture	[157]

Figure 2.26: Literature summary of materials, synthesis and release conditions for PPy based delivery systems (2/2)

2.3.2.3.B Effect of PPy counter-ions As presented in the previous section, the synthesis of PPy is affected by a lot of parameters, including the properties of the counter-ions to be inserted onto the polymer backbone. This results in a polymer with variable properties (electrical, structural, physical, etc.) and especially a different doping rate. It is thus logical that the release properties also depend on the doping anions and their characteristics. Even subtle variations in the counter-ion play a direct role in influencing the switching characteristics. A lot of studies have been done on the synthesis and liberation properties of PPy doped with model counter-ions. From these works, it appears that there are a restricted of main characteristics of the counter-ions that rule the comportment of redox properties; namely the type and size (and more generally the physical and electrical properties) of dopant counter-ions. We can also mention the effect of solvent-counter-ion coupling [57].

As presented before, the redox switching of PPy can lead to two extreme situations of exchange: anionic and cationic exchange. It has been shown in literature the important parameters ruling these different situations are on one hand the size of the counter-ions and on the other hand the composition of the electrolyte, coupled with the intrinsic properties of the PPy film. A great and widely used technique to study exchange properties in PPy is electrochemical quartz crystal microbalance (EQCM) which allows to measure weight variations at the order of the nanogram [104]. This technique can analyze in live the flux of ions in and out of the polymer film via frequency variations of the quartz crystal on which it is synthesized. Yet in 1987, Shimidzu et al. did an extensive study on the incorporation of various anions in PPy films by electropolymerization. They first showed that doping process was scarcely affected by the size of the electrolyte anion. Moreover, they evidenced that the oxidation/reduction process in the resulting PPy is largely affected by the size of the incorporated anion [105]. First, they showed that the relationship between the peak current of the redox process (i_p) and the sweep rate (v) shows peculiar changes with increasing the size of the incorporated anion. In the case of very small (Cl^-) or very large (polyvinyl sulfonate, PVS^-) anions, the linear relationship (i_p vs v) is maintained up to high values of v (figure 2.27). This means that the behaviour for these types of ions is a typical surface wave relationship. In the case of relatively small ions (benzenesulfonate, BS^-), the plot deviates from the linear relationship and involves a small part of the diffusion-controlled redox behaviour.

The contribution of diffusion-controlled redox behaviour was evaluated from the power *X value*, ie the slope of the linear relationship between $\log i_p$ and $\log v$, the X value reflecting the mobility of an electrolyte anion in the PPy matrix. $X=1$ corresponds to the redox reaction of a surface wave, the mobility of the electrolyte anion is thus higher than the electron transfer rate between redox sites. $X=0.5$ corresponds to the redox reaction of a diffusion-controlled wave, mobility of the electrolyte anion being lower in the PPy matrix. In PPy/Cl^- and PPy/PVS^- , an X value of 1 is found at all thickness while for PPy/TS^- the X value is dependent on film thickness, this proves the dependence of PPy with the anionic size. In fact, for different counter-ion characteristics (cations or anions), the X factor depends upon the molar mass of the species exchanged (1 for

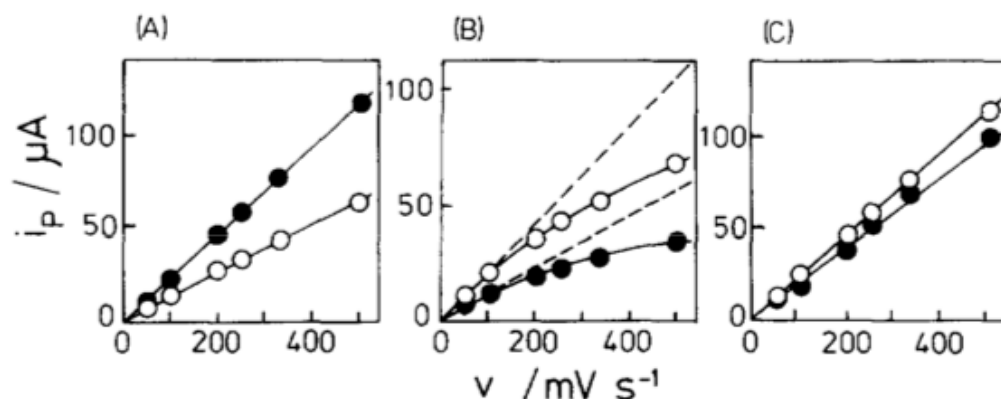


Figure 2.27: Relationship between i_p and v for the cyclic voltammograms of (A) PPy/Cl^- , (B) PPy/BS^- , and (C) PPy/PVS^- . (• — — — •) Anodic peak current; (○ — — — ○) cathodic peak current. Adapted from Shimidzu et al. [105].

Cl^- in PPy/Cl^- and for Na^+ in PPy/PSS^-) and between 0 and 1 for the other anion sizes, as can be seen on figure 2.28.

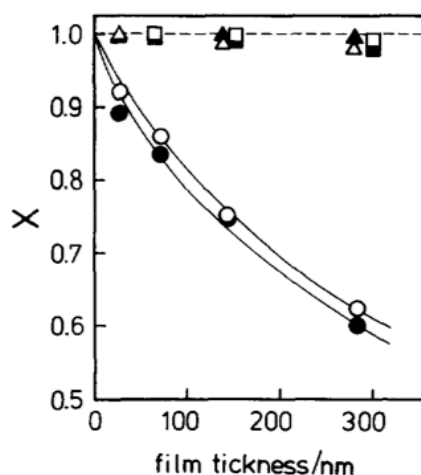


Figure 2.28: Relationship between X (power in $i_p \propto v^X$) and the film thickness of PPy/Cl^- ($\blacktriangle, \triangle$), PPy/TS^- ($\bullet, \circ$), and PPy/PVS^- ($\blacksquare, \square$). ($\blacksquare, \blacktriangle, \bullet$) Anodic peak current; ($\square, \triangle, \circ$) cathodic peak current. Adapted from Shimidzu et al. [105].

The observation of the X factor is thus an easy way to evaluate the mobility of counter-ions and observe their redox characteristics in function of their size. In this work, they also proposed a more general model for the redox behaviour of PPy with different counter-ions, by changing the films composition upon successive redox cycling in various electrolytes (see figure 2.29). This result was confirmed by the work of Kontturi et al. [106], by synthesizing polypyrrole films containing variable counter-ions of therapeutic activities (tosylate, salicylate, ...), all of medium

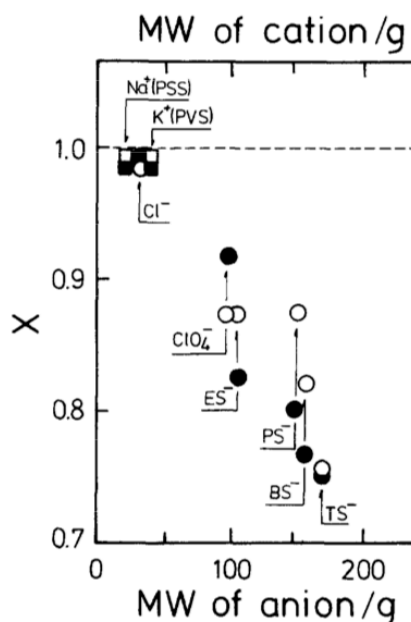


Figure 2.29: Relationships between X and M_W of the incorporated anion for PPy/SE (●,○) and M_W of the electrolyte cation for PPy/PE (■,□). (■,●) Anodic peak current; (□,○) cathodic peak current. Adapted from Shimidzu et al. [105].

size aromatic structures. Using EQCM and HPLC to follow the release of anions out of the films, they showed that the application of a reductive potential step leads to a variable ion exchange behaviour with time (or applied charge), as on figure 2.30. At the beginning, the mass of the film is increased revealing the entry of cations (associated with water molecules) inside the film. After a few seconds, the mass turns gradually in the opposite direction with the injected charge, indicating that the dominant process becomes that of the egress of anions from the film. A dependence on pH was also revealed. Moreover, a different counter-ion (naproxen) was proved to enter only in the surface pores-channels of the membrane and cannot penetrate further into the film. At this point, they thus proved that the amount of drug stored inside a polypyrrole membrane and the amount that can be released is dependent on the molecular size and structure of the drug.

The work by Naoi et al. confirms the models of either anion or cation exchange with small (BF_4^- , ClO_4^-) or large anions (PSS^- , PVS^-) [107]. In addition, they confirmed that films formed with medium-sized anions (TS^-) present both apparent anion and cation motion during the redox process, by comparing the mass changes during oxidation/reduction of the film with the predicted mass changes during the switching process. This latter prediction is based upon the proportion of anions to cations in the charge compensation process. The film thickness was kept sufficiently low so that Sauerbrey approximation could be used [108–110]. Also, redox sweep rate was low (10mV/s) to ensure the electrode system to be completely under thermodynamical

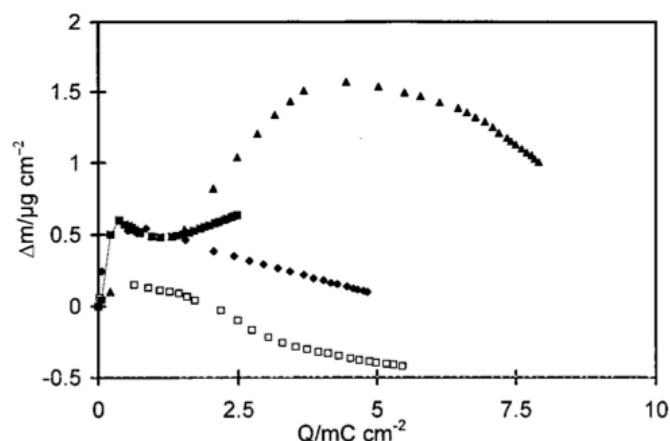


Figure 2.30: Mass change versus charge plot obtained during the reduction of a polypyrrole membrane loaded with different anions by a potential step from +500 to -500 mV versus SCE: tosylate ($\blacklozenge$), salicylate ($\square$), nicoside ($\blacktriangle$) and naproxen ($\blacksquare$). Adapted from Kontturi et al. [106].

control and considered free from kinetic or transport effects. During the synthesis of PPy/TS^- by cyclic voltammetry, Δm analysis show an increase in mass during oxidation of the monomer into polymer and its deposition onto the quartz surface. During the subsequent reduction, mass also increases at the reduction peak, indicating that cations are incorporated to the film to maintain neutrality. At lower potentials, mass decreases as an indication of anion movement out of the film. During the reverse scanning (re-oxidation), the decrease in mass is of lower magnitude than that are occurring during reduction, suggesting that both cations and anions were removed during the oxidation process. Figure 2.31 shows the variation of mass during polypyrrole synthesis (*ie vs* potential), the irreversible increase of mass indicates deposition of polymer on the electrode, while the redox (reversible) process demonstrates the incorporation of cations during the reduction reaction.

By plotting the mass changes, either measured by EQCM ($\Delta m(QCM)$) or estimated from transferred charge ($\Delta m(Q)$), they showed that even for small anions, there is a deviation between the two scheme of ion exchange. This is attributed to a process in which cations are inserted to compensate from anions which are trapped deep inside the bulk polymer and are not removed during reduction (assuming that mass change due to solvent motion is negligible). Medium size anions mixed release behaviour from polypyrrole was confirmed by Mirmohseni et al. [111, 112]. Moreover, the possibility to replace a certain counter-anion with another has been shown. They distinguished *outer-layer anions* (which can readily move in or out of the polymer and be replaced by solution anions) and *deeply-trapped anions* (which can hardly be replaced by solution anions).

The previous work done by Vorotyntsev et al. pointed already in this direction [113]. Starting from the general statement: *the main driving force for the mass change is an electroneutrality condition for the bulk film. A change in the film electronic charge must be accompanied by the*

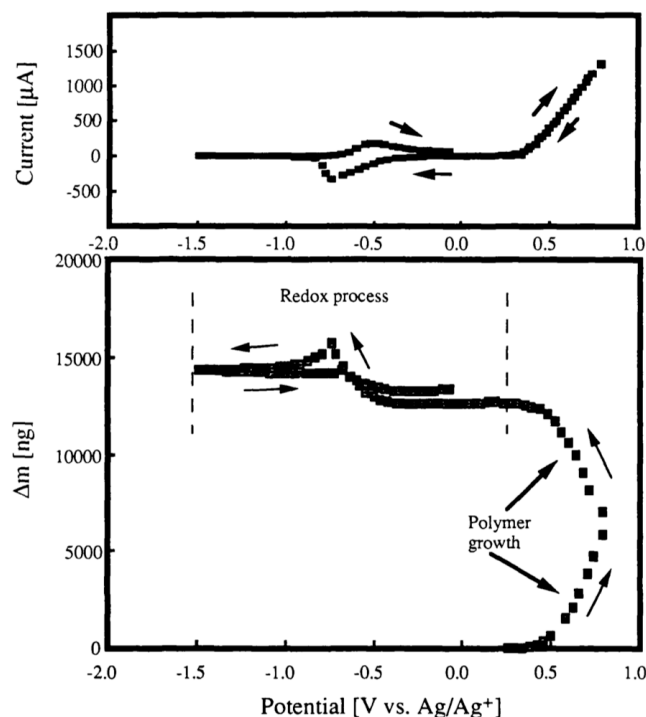


Figure 2.31: Typical cyclic voltammogram and mass change (Δm) for polypyrrole growth and redox process under cycled potential ($v = 10 \text{ mV.s}^{-1}$) in $(0.2 \text{ mol.dm}^{-3} \text{ TEATS} + 0.2 \text{ mol.dm}^{-3} \text{ pyrrole})$ in acetonitrile. Adapted from Naoi et al. [107].

equivalent charge of the ionic one leading to an exchange between the film and solution with charge species; they developed new theoretical approaches for the interpretation of EQCM data for the charging/discharging process in electron-conducting polymer films coated electrodes. The first proposed model treats ionic species as *free* mobile species, whose exchange with the solution may be retarded due to low values of the interfacial exchange constants.

A comparison of the limiting regimes of the model to experimental EQCM curves led them to reconsider some of the hypotheses inside the model, with the assumption that not all counter-ions accumulated inside the film at a high charging level can be expelled near their equilibrium potential. A possible reason of this might be some binding of anions to the polymer matrix. If cation insertion starts before anions removal, EQCM curve should change its slope at approaching electronic charge corresponding to the charge of the bound anions. Their second model takes into account the existence of two kinds of counter-ions, and assuming three types of ionic species inside the film, with possible kinetic limitations for their mutual transformation (*free anions*, *cations* and *bound anions*).

In this case, there are also 3 regimes in the charging-discharging process, depending on ion

properties:

1. *equilibrium*, which means a sufficiently rapid exchange of both free anions and cations with the solution ;
2. well separated processes of the free-anion removal and of the non-equilibrium cation insertion into the film (realized at low exchange rates for cations) ;
3. *fixed cations* (whose charge compensates that of immobilized anions).

In their work, Weidlich et al. [114] studied the effect of type, size and valency of the incorporated ions on exchange properties by EQCM, by synthesizing PPy with different doping ions (in size and charge) and cycling them in various electrolytes. The result can be summarized as a table (see table 2.2), as well as the corresponding considerations on the role of electrolyte. Nine cases are considered: PPy with univalent, bivalent or polyanions as dopant in combination with three electrolyte solutions composed of univalent or bivalent anions and univalent or bivalent cations. In the table, the exchanged species are indicated by an individual anion or cation, brackets indicate a weak(er) contribution. We can see that pure cationic exchange is found for polyanions as dopants in all cases, and also for PPy with small counter-ions and C^+ , A^{2-} in the solution. Pure anionic exchange appears for combinations of PPy with uni- or bivalent small CI and with anions of the same valency in the electrolyte solution. From this table, it is possible to present representative works based on the particular situations with selected counter-ions and variable electrolytes (and inversely). It is also clear that the role of doping ions can hardly be separated from the one of electrolyte, especially for the comparison of ions of different valencies. However, we can at this point underline the importance of this valency on exchange properties.

Table 2.2: Ion exchanger behaviour of PPy in dependence on counter-ions incorporated into the polymer and ions in the electrolyte solution. Adapted from Weidlich et al. [114]

	Counterion	Ions in electrolyte					
		1		2		3	
		C^+	A^-	C^+	A^{2-}	C^{2+}	A^-
1	(PPy/ A^-)	(C^+)	A^-	C^+	–	–	A^-
2	(PPy/ A^{2-})	C^+	A^-	–	A^{2-}	C^{2+}	A^-
3	(PPy/ PA^{n-})	C^+	–	C^+	–	C^{2+}	–

A^- : small monovalent anion/counterion, A^{2-} : small divalent anion/counterion, PA^{n-} : large polyvalent counterion, C^+ : small monovalent cation, C^{2+} : small divalent cation, (): weak ion exchange, –: no ion exchange.

The same conclusion was done in the work of Li et al. on polypyrrole films for ion exchange applications [115]. They defined two parameters to evaluate ion exchange behaviour: ion exchange rate and ratio. With these parameters, they compared polypyrrole films doped with different anions for both their anion and cation exchange abilities in different solutions. On

the effect of charge on exchange properties, Kepas et al. compared the properties of PPy doped with hexafluoroaminate and related systems. Electrodeposition, redox switching, ion transport/exchange, surface topography were studied [116]. They enlightened the differences in behaviour and linked them with the oxidation level, hindrance to the interfacial transfer of anions and a different contribution of oxygen/water redox couple.

As mentioned in the beginning of this section, in addition to EQCM measurements, another technique to separate various physical and chemical contributions to the electrode process is the electrochemical impedance spectroscopy. The common procedure is to construct electrical circuit models of electrode cell and thus characterize electrode process in terms of electrical analogs of the underlying phenomena and reactions [72]. Based on this, Kepas et al. observed that morphology differs with the doping ion, as well as the values of the components in the model circuits [116]. In the literature, a growing number of studies in the direction of size, charge and structure of dopant effects on PPy redox properties are done, confirming the general trends evoked sooner. The general statement that is to remember here is that the dopant effects on the liberation properties from PPy can be predicted from their characteristics. It also has been evidenced that these effects, even though sometimes isolable, cannot always be distinguished from other contributions. Moreover, the presence of dopant ions and their characteristics affect the other properties (film morphology, electrical properties, etc.).

2.3.2.3.C Effect of the electrolyte If we come back to the general statement at the beginning of this chapter, we see that transport properties can be separated into two extreme contributions from the opposite charges, anionic or cationic. The influence of electrolyte has already been evoked in the analysis of the counter-ion effects. In fact, both electrolyte and dopant effects are linked and it is delicate to separate each contribution. Although, in literature as well as in the present work, we can distinguish studies for electrolyte parameters influences on cationic transport on one side and anionic transport on the other side. The role of solvent can also be underlined. As a first statement, we can mention Zhang and Dong [117], who have shown the crucial importance of supporting electrolyte on switching properties, especially on the potential required to trigger the switching and the rate of switching. The solubility of ions in the solvent used and their affinity for polymer matrix determine switching properties [55]. Studies also proved media characteristics including pH, ionic strength, polarity and hydrophobicity to affect CP properties and the release of drugs [118]. In our case, it is of the greatest importance because the release aims to be *in vivo*. Media for release, even though mimicking body environment in general and nerve environment in particular, is an oversimplified model media. To extrapolate the comportment of the films for drug release in the implanted media, it is fundamental to study the effect of media on model drug release to estimate the variations that will occur from *in vitro* to *in vivo*.

In their work, Baker et al. [109] studied ion transport in PPy/PSS films for electrolyte with

increasing cationic mass by chronogravimetry and chronocoulometry. They established that amperometric and gravimetric responses observed for PPy/PSS in three sodium electrolytes are independent of the electrolyte type as expected under conditions in which both and only cations and water are the moving species. Changing the electrolyte system had a profound effect on both A-CV and G-CV responses. A lot of work has been done on cation-exchanger systems. In their study, Akieh et al. [119] analyzed the comportment of PPy/PSS films with electrolytes containing singly, doubly or triply charged metal ions. They showed first a small shift in the redox peaks while scanning in solutions containing different cations. Δm vs Q plots show two linear regions (see figure 2.32), as it was previously explained by Lien and Smyrl [120]. In their study on PPy/PSS films using QCM, they identified two regions of ion movement during redox cycling of the film in different electrolytes. By computing the slope of Δm against Q , apparent mass of ions involved in charge compensation during redox processes could be calculated. By this means Akieh proved the movement of solvent molecules along with cations during their insertion or removal. These water molecules are either free in the solvent or already bonded to metals ions (*ie* present as a part of an hydrated ion).

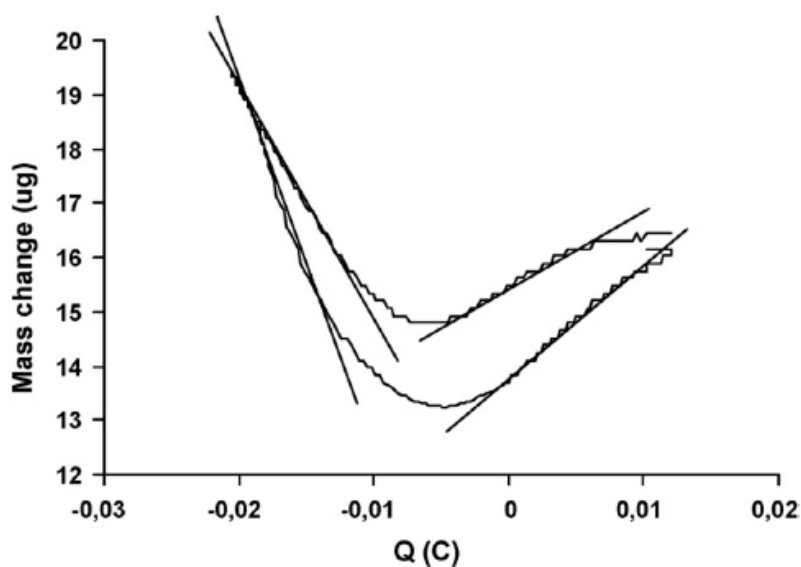


Figure 2.32: The mass change as a function of charge during redox cycling (3rd scan) of PPy/PSS in 0.1 M KNO_3 . Adapted from Akieh et al. [119].

In their study, Kim et al. [121] compared the exchange properties of DS^- and ClO_4^- doped PPy films by cycling voltammetry either in the same or different electrolytes. They emphasized the differences in electroactivity induced by the different electrolytes. On the analysis of small anion dopants, Bruckenstein et al. [122] explored quantitatively how the predominant ion transfer accompanying PPy redox switching depends upon charge type of the anion in presence in bathing solution. The predominant ion that undergoes the transfer shifts from anion to

cation upon replacing monovalent anions initially present in the film by divalent anions. The differences in the transfer (identity of ions and direction) create variations in the polypyrrole salvation characteristics, as a consequence of charge and size of transferred species via ion-bond solvent and/or via *free* solvent (*ie* change in volume constraints).

In general, the responses (associated with film population charges) of PPy films to redox cycling may be influenced by the identity of cations having the same fixed charge, by anion charge type and by redox switching history.

Compared to monovalent anion systems (ex. PPy/ClO_4 in $NaClO_4$), divalent systems (ex. PPy/ClO_4 in Na_2SO_4) exhibit a domination of cationic transfer during both oxidation and reduction. Three common features are attributed to divalent anion systems:

- the net mass change is negative (positive) for film oxidation (reduction)
- in all cases there is a hysteresis in the response of film population (mass) to film redox state (charge)
- there is a failure to restore equilibrated reduced state at the end of a redox cycle. Pure influence of electrolyte is confirmed and thus emphasize the importance of its choice or its implications for controlled release.

In their work, Iseki et al. studied the behaviour of PPy/TS films in aqueous solutions containing alkaline earth metal ions or tetraalkylammonium ions [123]. They demonstrated inactivation situations related to the interactions between cations entry in the films and anion release from it during the reduction phase. Another more recent study of Jim et al. focused on the exchange of pTS^- from PPy/pTS film to electrolytes of variable anionic and cationic composition. In NaCl, they observe both anionic and cationic representative redox peaks, saying that there are cationic (Na^+) exchanges at a certain potential and anionic (pTS^-) at another. Their observations confirms what can be found in literature.

The study on electrolyte anion re-incorporation has however been less studied. This is important in the scope of this work, because the history of the film does not end with the release of drugs, electroactivity of it has to survive the liberation.

2.3.2.3.D Effect of polymer characteristics Structural parameters of polypyrrole films also greatly affect release properties of PPy. The thickness of polymer is the most obvious parameter that has been studied. As a general constatation, studies found in literature concern films of relatively low thickness, because of the constraints related to EQCM interpretation (Sauerbrey model, viscoelasticity). Several people have shown that as film thickness increases, there is a corresponding increase in total amount of drug that can be released [106, 124–126] in films prepared with the same synthesis parameters. If we assume that drug loading is constant, estimation is that more polymer means more drug incorporated and so available for release.

However, the level of drug release is not linearly correlated with the increase of thickness in the film [127, 128]. This may be due to thicker films being less electroactive and changes in diffusion coefficient with thickness.

Inzelt et al. [52] described the effect of thickness of PPy films doped with small anions on exchange properties. They affirmed that the relative contribution of A^- and C^+ to the overall charge transport depends on the film thickness, even if small-size ions are used. In the case of thin films, C^+ expulsion accompanies oxidation, while for thick films the incorporation of A^- will be dominant at high positive potentials.

Although deviations from Sauerbrey model can be expected, magnitude of mass changes is reasonable, *ie* for thick films, mass changes accompanying redox transformation of the film are proportional to the dry thickness and the charge transferred. The same proportionality can be observed between frequency drop (deposition of the film) and film swelling, *ie* between frequency change related to the dry polymer and that of the polymer in contact with the solution. In previous studies [129], no dependence of ion transfer on thickness was studied. Explanations proposed could not explain thickness dependency. As an answer, we can mention the theory of metastable adsorption of de Gennes, which describes a density decrease of the polymer layer with the increase of distance from the electrode surface, concentration of charged sites also changes. They summarize it as follows : *if cation and anion transport could take place within the film in a concurrent manner, then cation transport was more significant for thin films, when a more compact structure predominates, so at least a qualitative elucidation of the observed phenomena can be given.* It should be emphasized that the motion of all mobile components (A^- , C^+ and solvent molecules) may occur at each potential, disregarding film thickness. A complementary or alternative explanation can also be given based on Feldbergs model [130]. In this model, it is suggested that the double layer capacity linearly increases with an increase of polymer converted into its oxidized (conducting) form. It may be assumed that mostly anions participate in the formation of the electrical double layer at potentials more positive than the potential of zero charge (E_{pzc}).

Kepas et al. used EIS spectroscopy to highlight the influence of thickness on the properties of PPy films deposited in successive layers [131]. They observed electrochemical charges to be scaled linearly with EQCM frequency changes recorded from electrodeposition. The conclusion is that we observe similar deposition efficiency, hydration, contribution of counter-ions and co-ions for each of two polypyrroles in the range of thickness studied. As a result, values of ratios of polymerization are meaningful for a comparison of thickness of the same PPy/electrolyte system. But they are dependent on the history of the film (number of cycles, electrolytes in contact, etc). They stated that the effect of film thickness and/or number of sublayers is more pronounced for anodic/mass injection. It should be kept in mind in the scope of this work that layer-by-layer deposition provokes an hindrance of charge transport in the polymeric electrode, as confirmed both by CV, EQCM and EIS. While charge passed during electropolymerization determines the

quantity of polymer formed, the rate of passing charge determines the speed of polymerization and subsequently the density and morphology of the film. A high polymerization rate form thick films with low densities, and inversely, for the same charge of deposition, low polymerization rates tends to create thin and more compact films [132]. Density of the film affects the diffusion of ions into the polypyrrole and the diffusion of electrolyte within the polymer.

2.3.2.3.E Effect of the electrochemical technique and parameters Electrochemical release of dopants requires injection of electrons to the polymeric film to achieve reduction process. The way of injecting these electrons depends on electrochemical techniques that are used. Moreover, the parameters of it rule the reactions of the film, such as rate of delivery, swelling/deswelling rate, etc. There exists a high diversity of electrochemical techniques with their singularities and parameters. In this thesis, we will focus on a small number of them, namely cyclic voltammetry and potential pulses or steps.

About cyclic voltammetry, parameters that can be changed are scan rate (speed of potential switching), potential range/limits and the number of cycles. At this point, we can enumerate some general trends observed for the previously mentioned parameters. Scan rate (speed of varying potential, dE/dt) is a direct way to control ion exchange. In their work, Bruckenstein et al. showed that PPy films exposed to sodium tosylate solutions change from predominant anion exchange mechanism to chiefly cation exchange behaviour when scan rate goes from 2 to 100mV/s [122]. Akieh et al. showed without any surprise that anodic and cathodic current peaks are lower when scan rate decreases [119, 133]. For PPy/PSS films at fast scan rate in KNO_3 , exchange mechanism was proven to be predominantly cationic (qualitatively and quantitatively). At lower scan rate (20mV/s) ion exchange mechanism was more complex. Values of apparent molar masses decreased with scan rates, as this trend can be seen in literature [54, 122]. At high scan rate, the movement of ions is kinetically favorable. However, these kinetic effects importance decreases with SR, since at low scan rates the dominant effect is the transport of neutral species, which have been explained to be the rate limiting step. Limits or boundaries of potential cycling as well affect rate of release. The higher the potential applied, the higher the quantity released. Furthermore, irremediable side reactions can appear at theses values of potential. For example overoxidation of PPy backbone in aqueous solution appears over 0.8V vs Ag/AgCl [134]. Besides, multiplication of CV cycles leads to the progressive modification of film properties, by changing its composition and swelling/deswelling repetition leading to physical (structural) degradation of polymer with the apparition of cracks. Delamination from the metallic electrode is finally appearing as the result of the destruction of it. Several researchers compared step potential drug liberation versus CV [103]. Thompson et al. proved CV to efficiently release at higher rates than potential or current pulses. In general, CV appears to be the most efficient method to release ions of choice [100]. When potential or current pulses were used, the contact with underlying substrate was maintained longer. Moreover, complexity for the fabrication of a CV-furnisher implantable device is by far higher than the one for pulses.

As mentioned numerous times before, redox processes do not occur instantaneously nor the accompanying inherent movement of ions. Nevertheless, chosen parameters for redox (both potential limits and times of application) largely outcome the effects and consequences of electrochemical methods on liberation of drug by polypyrrole.

In literature, the liberation of drug by the application of pulses is not widely described. The study of drug delivery by charge-balanced biphasic current pulses was done using in-vivo compatible devices [53, 157]. Nevertheless, the effect of the stimulation parameters on drug liberation on one hand, and on the integrity of the polypyrrole film on the other hand were not described. Moreover, this technique do not allow the constant control on the electrochemical state of the polypyrrole film. The application of potential pulses can overcome this problem. Currently, there is no report on the application of potential pulses for the delivery of drug from polypyrrole using an in-vivo compatible device.

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3 Effect of a model structuration at the nanoscale on a metallic electrode on the electrical properties and osteoblast cell behaviour

In this chapter is presented the topographical modification of an electrode surface at the nanoscale. Synthesis techniques and parameters for nanowire arrays formation with different characteristics (material, support and dimensions) are discussed. Afterwards, electrical properties are analyzed in terms of electrode impedance. Finally the effects on cell adhesion and growth induced by the modification of an electrode surface at the nanoscale are investigated. This work is submitted for publication.

3.1 Introduction

As explained in the objectives of this thesis, the first way to address interfacial issues is the physical (or structural) modification of the stimulation surface itself, in the aim of increasing the electrical stimulation performances of the metallic electrodes and the contact of living tissues with them.

Planar electrode materials (ex. untreated gold or platinum) have an impedance of around $1k\Omega.cm^{-2}$ at 10Hz. Porous materials have been tested to improve impedance properties, such as platinum black or Ti_3Ni_4 [1, 2]. Despite good performances, the blocking of the pores by proteins or other big molecules can lead to poor performances after a certain time. Recently, Kim et al. developed neural electrodes engineered with gold nanoflakes, which show good impedance performances [3]. One promising way to improve electrode performances stands in the creation of vertically aligned conducting nano-objects.

Nowadays, there exist many techniques to create arrays of aligned nanostructures composed of various materials. We can mention here the e-beam or focused ion-beam techniques, nanoparticle growth and template-based methods. Template-based methods offer the advantages of the great versatility in geometric parameters for the nanosized objects. In literature, a lot of examples can be found about the synthesis of vertical nanowires of multiple materials (metals, polymers, semi-conductors...). The common designation for this type of structures is *nanowire or nanopillar arrays* (NWA or NA) consisting in vertically aligned nano-scale cylinders with a defined density, distribution, shape and geometry.

The characterization of nanostructured electrodes is of tremendous interest because of the specific properties attributed to this kind of structures, especially at the scale studied in the scope of this thesis. Many techniques have been used to analyze, observe and characterize surfaces and link the observed architectures to their properties. We can distinguish for the purpose of this work three main tools used to investigate nano-featured surfaces and their properties in comparison to planar surfaces : microscopies (SEM, TEM, AFM) ; electrochemistry (CV, EIS) and numerical simulation.

Microscopy is the most reliable technique to clearly describe the exact architecture of observed surfaces. Among them, SEM has been widely used. Schröper et al. [4] describe a simple way of calculating the projected area for gold nanopillar arrays as in equation 3.1 using average values for height (h_{NP}), diameter (d_{NP}) and inter-pillar distance (x_{NP}) measured at an angle of 60° .

$$A_{3D} = A_0 + \frac{\pi d_{NP} h_{NP} A_0}{\sin 60^\circ (x_{NP} + d_{NP})^2} \quad (3.1)$$

Forrer et al. used TEM microscopy to obtain high magnification images of microtomed cross sections of nanowire arrays [5]. This allows them to reduce possible errors on the measurements and calculations of these mean parameters. The area calculated by microscopy is generally con-

sidered as the reference area for the determination of total *exposed* surface. It is this surface that will be compared with the ones found by other techniques. It should be noted the distinction between exposed area and active area, which could be different considering that all the exposed material(s) may not be equally electrically conductive.

However, transversal cuts as done by Forrer et al. are not always possible to observe and a significative error and undetermination can still remain. Moreover and more importantly, calculation of the total projected area and observation of geometry and structure of nano-featured surfaces is not the only determining analysis on properties of nanostructured arrays. The effect of these parameters on electrochemical behaviour is not straightforward and contains many informations about structural effects.

For stimulation devices, electrochemical properties are crucial, and comprise particularities which cannot be demonstrated solely by observation. Electrical current response of nanostructured electrodes is controlled by reaction kinetics, mass transport and the geometric topography of the nanostructure. To investigate gold nanowire arrays, Forrer et al. used cyclic voltammetry with a solution containing a well-known ferrocyanide redox couple as a reversible faradaic test system. In the case of diffusion-controlled faradaic reactions at nanowire arrays, the build up of non-linear diffusion profiles at the individual nanowires and their time-dependent overlap has to be considered for the assessment of voltammetric responses. This consideration can be represented as in figure 3.1.

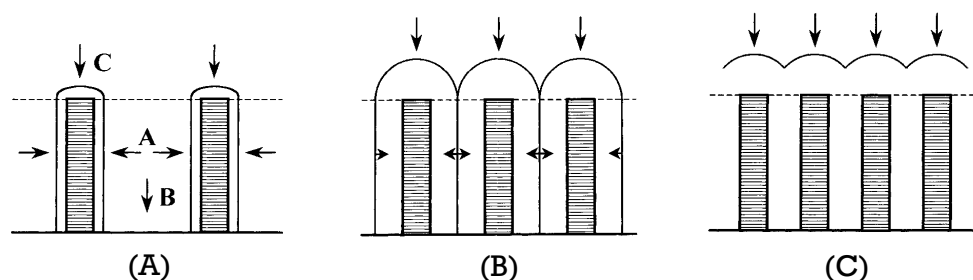


Figure 3.1: Schematic presentation of the diffusional conditions prevailing at nanowire array electrodes. (A) Individual non-linear case ; (B) complete overlap case and (C) limiting linear diffusion case. Adapted from Forrer et al. [5].

Considering nanowires as cylindrical elements, different situations are considered. Diffusional flux at each nanowire is controlled by :

- (A) approximately cylindrical diffusion at the lateral boundary of an ensemble of single nanowires
- (B) diffusion at the conducting base substrate between nanowires
- (C) diffusion at the top surface of pillars (which can be considered linear at initial short times and developping into approximately hemispherical diffusion afterwards)

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Under *individual non-linear case* conditions, total flux is the sum of all mutually independent fluxes at each nanopillar. This affirmation is valid as long as there is no overlap of the laterally expanding cylindrical diffusion layers between nanowires, *ie* at very short times after the onset of diffusional fluxes and at arrays with sufficiently large nanowire inter-distance. With increasing time, diffusion layers around each pillar expand to complete overlap and finally, a *limiting linear diffusion case* is reached. Diffusional flux in electrolyte volume elements between nanowires is completely exhausted, and transport only occurs between the front surface of nanowires and the adjoining bulk electrolyte in an approximately linear diffusion pattern.

Compared to (A) and (B), situation (C) corresponds to an electrode whose electrochemically active surface is reduced to the sum of nanowires top surface, in the interpretation of Forrer et al. The influence of pillars is thus dependent upon a certain number of parameters, including interspacing, concentration of the species in presence and time. The interpretation is controversial and will be interpreted carefully.

The first electrochemical parameter while studying electrochemical properties is the double-layer capacitance (C_{dl}), related to the residual current in the electrode. It can be calculated from the expression: $C_{dl} = |i_c|/\nu$, where $|i_c|$ is the average capacitive current and ν the scan rate. Electroanalytically exposed surface area A of nanowire array can be determined from C_{dl} either by considering the double-layer capacitance of the particular electrode-electrolyte system, or by measuring C_{dl} at an identical electrode with known exposed surface area. This measure gives the value of electrolytically active exposed surface [4–6].

Anandan et al. compared SEM and C_{dl} techniques to estimate active surface. They showed that SEM-calculated surface area is much smaller than the one by C_{dl} . A possible reason for this discrepancy is that C_{dl} estimation is based on the assumption that double layer capacitance is independent of the applied potential, likewise an electric capacitor. In electrochemistry, it is hardly the case [7]. C_{dl} technique for the calculation of active surface area is sometimes not a valid approach. Nevertheless, it is somehow a good estimation of it.

In order to evaluate the agreement between this value and the reality, an observed method of measuring C_{dl} can be employed. Since interface double-layer capacitance is proportional to electrode-electrolyte interface, one can use Electrochemical Impedance Spectroscopy (EIS). Moreover, values found by EIS are more likely to be reliable. Impedance behaviour is a reflection of electrical and electrochemical properties of the electrode materials and electrolyte solution, as well as microscopic morphology of the interface. Schröper et al. measured EIS spectra for planar gold electrodes and nanostructured ones (see figure 3.2). Electrode material and electrolyte solution are the same across all samples and the only significant difference is microscopic morphology. Therefore, it is the surface morphology that plays the most important role in causing differences in EIS spectra. At low-frequency limit of $|Z|$, impedance is lower for gold nanopillar electrodes in comparison to planar gold. For high frequencies, $|Z|$ is dominated by the uncompensated resistance of the supported electrolyte. The value is thus logically the same for both electrodes.

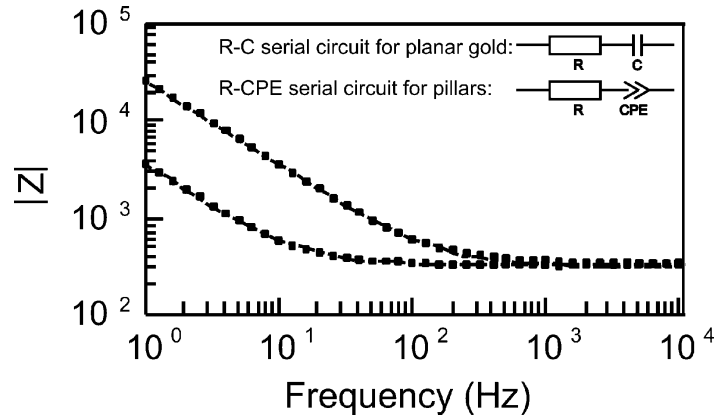


Figure 3.2: Impedance spectroscopy of nanopillar (lower curve) and planar (upper curve) gold electrodes measured in 0.15 M NaCl. The lines through the data points correspond to the fitted results. Inset shows equivalent circuits: R-CPE serial circuit model for nanopillar electrodes and R-C serial circuit model for planar gold electrodes. Adapted from Schröper et al. [4].

In order to analyze quantitatively the changes in active surface, data were fitted with circuit models representations in order to determine the interfacial double layer capacitance for both electrode types. For planar gold, equivalent circuit is a simple R - C circuit while a R - CPE circuit is used for pillars. CPE component is used to take into account electrode particular comportment. A constant phase element (CPE) is a non-intuitive element used to represent the inhomogeneous characteristics or some dispersion of a physical property of the system. Its mathematical representation is given by : $1/|Z| = Y = Q_0(j\omega)^n$, where Q_0 is the admittance ($1/|Z|$) at $\omega = 1 \text{ rad/s}$. At $n = 1$, Q_0 is equal to C (pure capacitance). One physical explanation for the necessity for CPE is electrode roughness causing deviations from pure capacitance. Other models can be proposed, as the $R(RQ)$ model of Cui et al. with a R_T resistance in parallel with a CPE .

By definition, capacitance is proportional to the surface area of the electrode, therefore Q_0 can be used to compare surface area. Cui et al. [8] previously explained the length scale dependency of the effective surface area. For impedance signals, surface area is a function of temporal frequency of the signal : high-frequency signals sense a smaller effective surface area. At low frequency (1 rad/s) the appropriate characteristic length scale for measuring surface area is the size of hydrated ions. The higher Q_0 , the higher effective surface area.

Based on the fitting of impedance data on Bode plots, effective surface area increase factor of Schröper nanopillar array was found to be twice the one based on CV measurement in NaCl electrolytes. But this still remains different than the SEM measured one. Same conclusions were done by Yoon et al. where the calculated impedance of nanoelectrode is lower than the one of planar electrode. The inconsistency stated by Schröper et al. forced researchers to evaluate electroactive surfaces with other techniques. The usual technique to link measurable

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electrochemical parameters to active surface is to use a redox probe relation that links height of the current peak to cyclic voltammetry parameters. Forrer et al. used the reversible redox system $Fe(CN)_6^{4-/3-}$ on various nanowire lengths and a flat gold surface as a reference (identical geometric surface area). Heights of faradaic redox peaks at nanowire arrays are noticeably lower than redox peaks recorded for flat gold electrode, corresponding to the surface of the top of pillars. They conclude that only limiting linear diffusion case applies under the experimental conditions of faradaic reaction.

Anandan et al. [6] studied the CV dependency of current peak on plane Au electrode and gold nanopillar array electrode in ferrocyanide solution. If the electrochemical process is a linear-diffusion driven process (*ie* in the semi-infinite linear diffusion case), the peak current (i_p) can be related to the active surface (A) and the square root of scan rate ($v^{1/2}$) by the *Randles-Sevik* equation (3.2).

$$i_p = (2.6910^5) n^{3/2} ACD^{1/2} v^{1/2} \quad (3.2)$$

where n is the number of electrons, A is the electrode active area, C is the bulk concentration of reactive species, D is the diffusion coefficient and v the scan rate.

The evolution for current versus $v^{1/2}$ follows a first order tendency with a slope higher on nanopillars. This suggests that electrochemical behaviour is indeed governed by a linear diffusion mechanism. Moreover, plots of amperometric current versus concentration of ferrocyanide in the solution also show linear tendency, with a higher sensitivity of nanostructured electrode, which is attributed to the increase of surface area. The small increase of electroactivity for nanostructured surfaces found by Schröper et al. are interpreted with an additional charge transferred by the redox process within the upper electrolyte elements between nanopillars [4]. More recently, Ramulu et al. confirmed this trend by studying the electron transfer process of ferricyanide through bare gold and gold NWA electrodes [9]. They attributed that to the larger surface area of the three-dimensional array type wires, which can increase the active surface area. This was already observed by Wang et al. [10]. Moreover, Lin et al. showed that a bare Au electrode surface, linear diffusion occurs, while at the nanostructured electrode surface, 3 dimensional radial diffusion occurs [11]. In addition, Zhao et al. demonstrated the vertically aligned nanowires on electrode surfaces can function as an *electron antennae* and enhance the electron transfer rate by promoting the electron transfer between the redox couple and the electrode [12].

On the other hand, a measured electroactive surface which is smaller than (or equal to) real 3D surface can be explained by the assumption that the electrolyte volume elements between nanopillars are exhausted within a short time. System is proved to be a diffusion-limited system by plots of current versus scan rate. Schröper et al. underlined the fact that previous methods for the analysis of gold nanopillar surface area increase are based on diffusion controlled redox systems and capacitive effects. They produce inconsistent results and are not fully concordant

with the geometrically determined values.

To overcome this limitation, surface-bound redox systems were used to determine the actual surface area of nanopillar arrays, based on gold oxidation or immobilized compounds. Schröper et al. used surface-bound redox systems (immobilized ferrocenylhexanethiol (FcHxt) and immobilized cytochrome c (cyt c)), and oxidation/reduction of gold to compare the nanopillar arrays electrochemical responses with planar gold electrode surface, which served as reference system [4]. For example, the preparation of an homogeneous layer of cyt c molecules (redox proteins with a reproducible and reversible redox chemistry) on the gold surface is done via the electrostatic adsorption of carboxy-terminated self-assembled monolayers (SAM) and its interaction with the positively charged lysine residues on cyt c. The comparison of redox peaks obtained by cyclic voltammetry on cyt c covered flat gold and nanostructured gold surfaces lead to the calculation of the surface area increase factor induced by the pillars.

On the other side, the gold oxidation approach is based on the anodic oxidation/reduction of the surface gold atoms. The potential range is chosen as described in the literature to prevent the oxidation of the subjacent bulk phase. By the integration of the cathodic peak area obtained during a CV experiment, the charge density for reducing the Au-oxide ($\sigma_{red(exp)}$) was obtained and compared to the theoretical charge density ($\sigma_{red(theor)}$) of a known Au surface area.

For these systems, it is worth remarking that the electroactive surface area is at maximum equal to the 3D surface area determined by SEM, because of passivation effects that can occur as a consequence of contamination or barrier layers remaining from the fabrication process. These methods gave results in good agreement with geometrical method although not identical.

On the other hand, nanoscale structure variations can have influences over the biology of tissues in contact. Actually, in addition to the study of electrochemical performances, it is interesting for the comprehension of the contact between stimulation electrodes and the implanted tissue to investigate the effect of the structure at the scale of the cells and under. This contact influences both the occurrence of inflammatory reaction and the future performances of stimulation, via the adhesion and (re-)growth of cells onto the stimulation surface or their distance to the electrical stimulation parts, for example.

As von der Mark states about the biomimetic surface modifications at the nanoscale: *a surprising consequence of the nanoscale engineering of the surface of biomaterials is the finding that, by controlling the topography alone, the type of cellular responses can be directed in different directions* [13]. Biological systems are highly sensitive on specific features. At the nanoscale, surfaces are rarely totally flat, as the process to create or modify them (and the implant that they are part of) often engender the appearance of a certain roughness. Corresponding reactions of cells in contact with those surface are thus a consequence of it. With the growing interest to nanotechnologies on both material and biological point of views, it is crucial to understand the effects of the creation of nanofeatured objects on the aim of controlling biological reactions by this mean.

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Literature about direct influence of topography (especially at a nanoscale) and the particular effects of a restraint number of parameters on cell behaviour (as a first step within the road to implantation) is wide and furnished. Nevertheless, it is even actually not possible to understand clearly the importance of each parameter, on one hand because of its interlacing with other modifications and on the other hand because of the - actually decreasing, but still great - difficulty to create model structures at a nanoscale.

A first noticeable fact is that the reaction of cells to peculiar topographies depend on the scale of it, *ie* micron sized topography does not engender the same reaction as nanoscale topography. Though chemistry and topography interact in a complex synergetic manner, it has been shown that topography by itself is able to influence the behaviour of cells in contact [14–18]. Several techniques have been reported in the comprehension of cell-surface interactions, such as electrostatic and plasma spray, as well as physical vapor deposition (PVD) techniques like electron-beam evaporation and deposition. Micron-scale topographies have been designed on implants since a long time, resulting in improvements of the insertion into biological environments. Giving the fact that implants are exposed to complex biological environments, they are in contact with multiple cell types. Those different cell types may not respond in the same way to the designed surface cues, and the optimization of implant surfaces will head to different levels of topography.

Over the past few years, the fabrication of substrates of micro- and nanometer scales which replicate the structure and length scale of certain native topographies in two-dimensions have been created following the developments in advanced engineering techniques [17]. Cell responses to topographical cues depend upon a lot of factors : cell type, topographic geometry and size, physical properties of substrate, ... [19]. Rapidly growing literature conveys a lot of studies carried out about the effects of nanotopography, with a specific interest on cell morphology, attachment and adhesion, proliferation and migration, and complex biological responses such as genotypic alteration, differentiation or cell-cell interaction.

The exact understanding of the connection between nanoscale surface designs and cell response is still not transparent. General trends can however be established.

First, protein adsorption is considered as a key step in the process of the subsequent cell adhesion on implants, and is as well influenced and partially controlled by nanoscale topographical modifications [16–18, 20]. With nanostructuration comes the increase of surface area, and the alteration not only of the distribution but also on the amount of protein adsorption [20]. Even though variable, evidence have shown that protein adsorption is often higher on surface with the nanoscale structure compared with the smooth counterpart. Moreover, when nanofeatures get closer to protein molecular dimensions, modifications of protein adsorption may occur [21]. Surface wettability may as well be changed, affecting protein adsorption and conformational configuration.

As mentioned before, various techniques have been used to create micron scale topography on the surface of biocompatible polymers or metals for implant modifications. Theses features are

shown to effectively enhance in vitro and in vivo biological events [17, 18, 20, 22].

Evidences that nanoscale surface properties influence biological process and cell behaviour encourage the creation of nano engineered surfaces with controlled features. For most cell types, it has been shown that the presence of micro- and nano- grooves lead to the alignment and elongation of cells in the direction of grooves [17]. The depth and width of these grooves and the cell type however influence the degree of their morphological changes. Other nanofeatures, such as pits, pillars and nanotubes with variable shape, size, density and height have also a unique influence on cell behaviour [15, 22–25].

Both polymeric and metallic pillars have been investigated, together with the effect of their geometry and spatial distribution. Baker et al. studied the effect of PDMS pillars height and spacing on the attachment and proliferation of fibroblasts and macrophages [22]. They showed cell-type dependent results, in addition to an effect of structural parameters. The presence of pillars was found to reduce adhesion, spreading and proliferation of macrophages, especially in the presence of a high density of pillars, whereas attachment and proliferation of fibroblasts was substantially enhanced by increasing pillars height.

The pillar structure was proved to be of interest with neuronal cells too. Xie et al. showed that neuron mobility can be restricted by placing them in contact with nanopillars [26]. The growth pattern of neurons on pillars does not differ from the one found on flat surfaces. However, they observed that neurons not only survive on the top of pillars, but preferentially attached to them. Besides, adhesion, proliferation and viability of neuronal cells can be influenced by other kinds of nanostructures. By studying the growth of PC12 cells on poly(ϵ -caprolactone) nanowires, Bechara et al. showed that cells interact with nanostructures through an extensive network of cell lamelopodia and filopodia [27].

Metallic nanopillar structures were studied, especially to improve the monitoring and stimulation of cells. Brüggemann et al. synthesized gold nanopillar arrays to measure the extracellular potential of cardiac muscle cells [28]. They showed that the impedance of such nanostructures is lowered and interaction with cells is improved along with the amplitude of recording. For neurite development of PC12 cells on metallic nanowire arrays, Haq et al. showed that the presence of a dense nanowire array leads to higher cell density and shorter neurite extension [29]. In the perspective of stimulation implants, the nanostructuration of metallic surfaces offers numerous advantages (lower impedance, higher active area, etc.) in addition to the structural influence on cell activities.

In this thesis, an approach in two times was first followed on the material aspects. Various nanowire arrays have been synthesized, with different materials and geometrical characteristics. Because platinum will be used in the final application of the cuff electrodes, the first study was done on the fabrication of platinum nanowire arrays. In a second time, the synthesis of gold nanopillar was studied in order to analyze the electrochemical properties of nanowire arrays of homogeneous material composition. The synthesis conditions were optimized in order to obtain the desired characteristics. The 3D surface area and geometry of the electrodes was determined

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by SEM. These results were compared with cyclovoltammetric and impedance studies as well as to obtain informations on the electrical properties at the solid/liquid interface. Afterwards, we focused on the biological influences, by investigating the effect of gold nanopillars on pre-osteoblast cells behaviour in short term in vitro culture. The choice of pre-osteoblastic cell type was motivated on one hand by the opportunity to apply the experience developed in the lab, and on the other hand by the possibility to compare the results with a more widely furnished literature on this peculiar cell type. Actually, this kind of structural modification is currently being developed in the field of bone implants and tends to emerge in the field of neuronal implants. In this work, the use of a *model* cell type is a first step towards the systematic analysis of the influences of metallic nanopillar arrays dimensions on the tissue in contact.

These nanostructured surfaces are prepared by the same hard-templating electrochemical method than before, leading to the creation of gold nanopillar arrays with well-controlled dimensions (diameter, height and density). The use of such a fabrication technique to create a nanostructuration of controlled dimensions aims at investigating the influence on cell behaviour of structural changes solely (*ie* in absence of surface chemical composition changes). Two densities of gold nanowires presenting approximately similar pillar height and diameter were prepared, and osteoblast cells were grown on these nanostructured gold surfaces. Flat gold surfaces were used as control. Scanning electron and fluorescence microscopy were used to characterize cell attachment, morphology, cytoskeleton changes and focal adhesion formation, whereas cell metabolism was studied using alamar blue assay.

3.2 Materials and methods

3.2.1 Preparation of platinum nanopillar arrays

Platinum nanopillar arrays were prepared by an electrochemical template method (see figure 3.3). The electrochemical growth of Pt nanowires was carried out in a home-made one compartment Teflon cell at room temperature with a Pt disk counter-electrode and an $Ag/AgCl/sat. KCl$ reference electrode (*ChInstruments*). The electrosynthesis was controlled using an *EGG Princeton Applied Research 273A* potentiostat / galvanostat. Nanoporous polycarbonate (PC) track-etched membranes with a thickness of $21\ \mu m$, pore diameter of 150 nm and a pore density of $10^8\ cm^{-2}$ were supplied by *it4ip* [30] and used as templates. A 450 nm gold layer was previously evaporated on one side of the membrane, serving as working electrode. The Pt nanowires were electrodeposited potentiostatically on disk samples (Area = $0.282\ cm^2$) at 0 V from a homemade solution of $0.01\ M\ Na_2PtCl_6 \cdot 6H_2O$ and $0.5\ M\ H_2SO_4$ (*Acros Organics*) in de-ionized water. The amount of material deposited within the nanopores was controlled by the total electrical charge passed during the electrodeposition process. After deposition of the Pt nanowires, the PC template was removed by dissolution in dichloromethane. The brush of Pt nanopillars attached to the gold surface was finally rinsed with a $0.5\ M\ NaOH$ (*Acros Organics*) solution in order to

remove any trace of PC onto the nanowires.

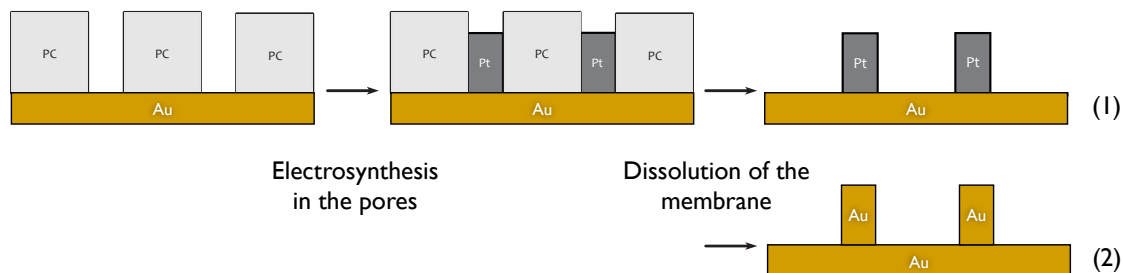


Figure 3.3: Diagram of the hard template method for the preparation of Pt nanopillar arrays on a gold substrate (1) and Au nanopillar arrays on a gold substrate (2).

3.2.2 Preparation of gold nanopillar arrays

The surfaces with gold nanopillars were prepared by an electrochemical templating method, carried out in a home-made one compartment Teflon cell at room temperature, with a Pt disk counter-electrode and an $Ag/AgCl/sat.KCl$ reference electrode (*ChInstruments*). The electrosynthesis was controlled using an *ChInstruments Chi660B* potentiostat. Gold-coated Si wafers covered with a $1\mu m$ thick nanoporous polycarbonate layer with pore diameter of ~ 100 nm and pore density of $10^9 cm^{-2}$ were supplied by *it4ip* [www.it4ip.be] and used as templates. The Au nanopillars were electrodeposited by cyclic voltammetry ($[0.8 - 0V]$ vs $Ag/AgCl/sat.KCl$, $100mV/s$) into the pores on a disk area of $0.25 cm^2$ from a solution of $0.027MKCl + 0.027MK_2HPO_4 + 8mMKAuCl_4$ in de-ionized water. The amount of material deposited within the nanopores was controlled by the number of CV cycles (15 or 45CV). After deposition of the Au nanowires, the PC template was removed by dissolution in dichloromethane for 1h (*Chem Lab*) and in a 0.5 M NaOH solution for 1h (*Acros Organics*) in order to remove any trace of PC from the nanopillars. All chemicals were purchased from *Sigma-Aldrich* (Belgium) and used as received. For cell culture experiments, gold-coated Si wafers covered with a $1\mu m$ thick nanoporous polycarbonate (PC) layer with a pore diameter of 100 nm and pore densities of respectively, $1 \cdot 10^8$ and $4 \cdot 10^9 cm^{-2}$ were supplied by *it4ip* (www.it4ip.be) and used as templates. The Au nanopillars were electrodeposited by cyclic voltammetry (CV : 0.8V to 0V vs $Ag/AgCl/sat.KCl$, scan rate = $100 mV/s$) into the pores on a disk area of $0.46 cm^2$ from a solution of composition shown in 3.3, in de-ionized water for low (high) density nanoporous membranes, respectively.



The amount of material deposited within the nanopores was controlled by the number of CV cycles (3 and 10 cycles for the $1 \cdot 10^8$ and $4 \cdot 10^9 cm^{-2}$ densities, respectively).

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3.2.3 Scanning Electron Microscopy

Gold surfaces were observed by scanning electron microscopy (SEM) using a high resolution LEO 982 field emission scanning electron microscope (FE-SEM, Carl Zeiss SMT Inc.), operating at 5 kV. Pillar density is considered equal to pore density of the used template, as pore filling rate is assumed to be close to 1. Mean height and diameter of nanopillars was calculated from 50 pillar length measurements (from 5 different random areas) on 5 samples tilted at an angle of 30° to the vertical, using *ImageJ* image analysis software (version 1.45i). Measured height (h_m) is linked to pillar height (h_p) by the relation $h_p = h_m / \cos 60^\circ$.

Cell interaction with flat gold and nanopillars was also analyzed using SEM. Cells were fixed with 3% glutaraldehyde (*Sigma-Aldrich*, Belgium) for 30 min at 4°C. Samples were then washed 3 times with PBS and were gradually dehydrated by successive immersion for 5 min. in 20, 40, 80 and 100% ethanol each for 5 min. Samples were kept covered with hexamethyldisilazane (HMDS, *Sigma-Aldrich*, Belgium), after which samples were left drying overnight in fume-hood. To avoid charging effect, the samples were coated with a 5 nm thick chromium layer prior to SEM analyses. For quantitative analysis of cell morphology, images were captured from triplicate samples (5 random fields each). Cell morphology was quantified in terms of mean cell area and circularity using *ImageJ* (version 1.43u) image analysis software, on a given number ($n = 100$) of randomly chosen cells. Cell circularity, which is used to describe cell shape, is equal to $4\pi \cdot \text{cell area} / (\text{cell perimeter})^2$. A value of 1.0 indicates a perfect circle while a value of 0.0 points to a completely elongated polygon.

3.2.4 Electrochemical analysis

All voltammetric experiments were performed with an *ChInstruments Chi660B* in the home-made three-electrode cell arrangement used for synthesis, at scan rates between 10 and 100 mV/s without compensation of the electrolyte resistance. A spring-like Pt wire (23cm, *Bio-Logic*) and an *Ag/AgCl/3M NaCl* reference electrode were employed as counter (CE) and reference (RE) electrodes, respectively. All chemicals were received from *Sigma-Adrich* and were of high purity grade (used as received without further purification). All aqueous solutions were made using ultra-pure water produced by a Millipore MilliQ system, and purged with Nitrogen before use.

3.2.5 Electrochemical Impedance Spectroscopy

Impedance characterizations of gold flat surfaces and nanopillar arrays were performed using a *ChInstruments Chi660B* potentiostat in the same three-electrode cell arrangement as for synthesis. An *Ag/AgCl/3M NaCl* electrode (*Bio-Logic*) and a spring-like Pt wire (23cm, *Bio-Logic*) were used as RE and CE, respectively. The amplitude of the ac voltage was 5mV. The data measured in 0.1M NaCl or 5mM $K_4Fe(CN)_6$ + 0.1M KCl were analyzed with the software

ZView (Version 3.3a, *Scribner Associates Inc.*) using a *R-CPE* circuit, consisting of a resistance (*R*) together with a constant phase element *CPE* [4]. The CPE was chosen because of the irregular (3D) structure of the nanopillar surface. For reference analysis and fitting of planar gold surfaces, the same circuit was used. All impedance measurements were carried out at a frequency range of 10^5 to 1Hz, at 0.6V vs reference (applied for 10s before and during measurements).

3.2.6 Cell culture

MC3T3 preosteoblasts (MC3T3-E1 Subclone 14, product number CRL-2594TM, from the American Collection of Cell Culture, *ATCC-LGC Standards*, France) were cultured in α -MEM medium (*Invitrogen*, Belgium) supplemented with 10% of foetal bovine serum (*Harlan*, Loughborough, UK), 1% sodium pyruvate (*Sigma-Aldrich*, Belgium) and 1% penstrep (*Invitrogen*, Belgium). Cells were maintained by passaging them every 3 days. Flat gold and nanopillar samples were sterilized by immersing them in ethanol for 30 min. Samples were then washed 3 times with phosphate buffer saline (PBS) prior to cell seeding. Cells (10,000 cells per sample) were seeded on flat gold or on gold nanopillar samples (sample surface : 1 cm^2).

3.2.7 Fluorescence microscopy

At the end of the cell incubation time, samples were washed twice with PBS and fixed with 4% paraformaldehyde for 10 min at room temperature, followed by three times rinsing with PBS. Permeabilization was carried out using 0.1% Triton X-100 in PBS, for 5 min at RT. The samples were then rinsed twice with PBS and incubated in 1% BSA in PBS for 30 min, then with primary anti-vinculin mouse monoclonal antibody (Millipore, Overijse, Belgium) diluted in 1% BSA (1:500) for 1h at RT. Samples were washed three times with washing buffer (0.05% Tween 20 in PBS). Cells were stained with secondary goat anti-mouse FITC- conjugated antibody (1:200, *Millipore*, Overijse, Belgium) and Alexa Fluor 594 phalloidin (*Invitrogen*, Belgium) for 30 min at RT. Samples were mounted over glass coverslips using Vectashield mounting medium containing DAPI (*Vectors Laboratories*, Burlingame, USA). For fluorescence microscopy, a *Leica DM RA 2* microscope equipped with a *Leica DFC 300Fx* camera was used. All images were processed and scaled using the *Leica Q Win* software (Version 3.0).

3.2.8 Alamar blue assay

The cell metabolic activity was measured by using Alamar Blue (resazurin-based) assay (*Sigma-Aldrich*, Belgium). Reduction of the dye by living cells decreases the amount of its oxidized form (blue) and concomitantly produces a fluorescent intermediate product (red), whose amount can be linked to cell metabolic activity [31, 32]. A 0.05% of resazurin dye solution was added to cells cultured for a given time on samples, and incubated for 2h at 37°C. 200 μ l of cell media

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was then transferred to flat bottom 96 well-plates and fluorescence intensity was measured using a fluorescence *SpectraMax Gemini XS* plate reader (Molecular Devices) at an excitation wavelength of 540 nm and an emission wavelength of 590 nm. The results were acquired using *SoftMax Pro40*, a software provided with the equipment.

3.2.9 Statistics

All error bars on data are expressed as standard errors from the mean. The statistical significance was determined at 95% level by a one-way *ANOVA*. Post-hoc statistical analysis of the means of individual groups was performed using Tukeys test. * represents $p=0.05$, ** represents $p=0.01$ and *** represents $p=0.001$, statistical significance was reported with relation to flat gold sample in all the cases, unless otherwise stated.

3.3 Results and discussion : effect of the structuration at the nanoscale on the electrical properties of a metallic electrode

3.3.1 Platinum nanopillar arrays

Platinum nanowire arrays were synthesized during the early steps of this thesis, as a proof of concept of nanostructured electrodes for the local and controlled delivery of dexamethasone [33]. The efficiency of the developed process, based on the hard templating method, for preparing nanostructured electrodes was checked by analyzing the electrode surfaces at each step of the preparation using SEM. Parameters to characterize a nanopillar array are : density (d), (mean) pillar diameter (Φ_p) and (mean) pillar height (h_p).

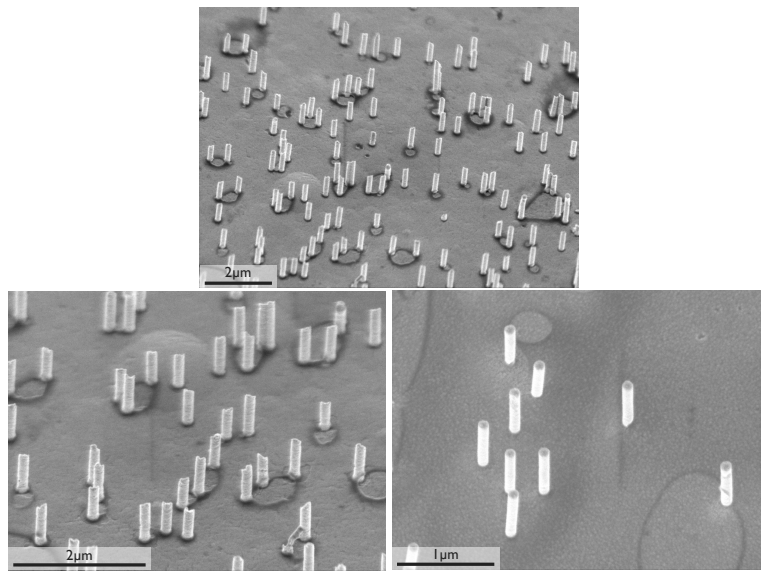


Figure 3.4: SEM pictures of typical brushes of self-standing Pt nanopillars on a gold surface ($Q_{Pt} = 24 \text{ mC/cm}^2$, $\Phi_p \sim 150 \text{ nm}$, $d = 10^8 \text{ cm}^{-2}$)

First, a nanopillar brush was synthesized by electrodeposition of platinum within the nanopores of a PC membrane. Figures 3.4 show typical features of a brush of self-standing Pt nanopillars obtained after dissolution of the PC template. We can mention here that the base of the electrodes is composed of gold, because the technique to synthesize a nanoporous membrane on a platinum electrode is not yet fully controlled. As expected, diameters of the nanopillars are quite monodisperse and with diameters corresponding to the pore diameter of the template used ($\sim 150 \text{ nm}$). In this configuration, *ceteris paribus*, the only nanopillar array characteristic that can be changed by the electrochemical process is the mean pillar height (h_p). As mentioned before in the literature for the chronoamperometric synthesis of nanostructures, the wire length can be controlled either by the electrical charge passing through the system during the synthesis [34] or by the time of the application of potential [35, 36]. Actually, both correspond to the same trend.

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In general, it is more accurate to use the charge as a control of the nanostructure dimensions. Indeed, the time needed to synthesize a certain structure depends not only on the dimensions of the structures to create, but also on the concentration of the synthesis solution and on the applied potential.

Using electrical charge as the control parameter is the easiest way to predict the mean pillar height, taking into account the geometry of the pores. In this work, the potential was automatically turned off when the desired charge was reached. For the templates used in this first study (PC membranes with pore diameter of 150 nm and pore density of 10^8 cm^{-2}) the mean pillar height can be related to the electrical charge by the following empirical relation: 15.5 mC/cm^2 for an $h_p = 500 \text{ nm}$, based on SEM and TEM measurements. An empirical formula can be generated for the general expression of mean pillar height:

$$h_p = k \cdot \frac{Q}{S \cdot \Phi_p^2 \cdot d} \quad (3.4)$$

where k is a constant linked to the synthesis parameters (concentration, material, potential), Q (mC) is the electrical charge, Φ_p (nm) the pore diameter, d (cm^{-2}) the pore density and S (cm^2) the sample surface.

The growing on the electrode substrate of a Pt nanopillar brush with such structural characteristics ($d = 10^8 \text{ cm}^{-2}$, $\Phi_p = 150 \text{ nm}$, $h_p = 500 \text{ nm}$) leads to an increase of $\sim 25\%$ of the active electrode surface. One serious advantage of our proposed method for preparing nanostructured electrodes, compared to the recently reported method based on the use of self-assembly of polystyrene nanobeads as template [37], is that here the increase of active surface area can be adjusted by playing on the different structural parameters of the brush. Indeed, the pore diameter of the used template can be fixed between 30 and 200 nm and the pore density between 10^6 and $5 \cdot 10^9 \text{ cm}^{-2}$ [30]. The height of the nanopillars can be tuned between only a few nanometers to a few microns. Though, to avoid the breaking of the Pt nanopillars when removing the PC template, their *aspect ratio* (height/diameter) must be kept below ~ 4 , based upon observations. It is important to remember that this ratio is higher when the material of nanopillar is the same as the one of the bottom layer. Also, diameter of pillars is of importance in the possibility to obtain high aspect ratio.

The possibility to cover these nanostructured electrodes with conducting polymer and use them to release a molecule of interest will be explained later. At this point, what we can point out is the possibility to control the parameters of a nanostructured electrode to create a model nanowire array of a chosen material. In the next section, the properties of a model gold nanowire array will be studied, in terms of active surface and electroactivity.

3.3.2 Gold nanopillar arrays

3.3.2.1 Structure analysis by microscopy

Synthesis of gold nanopillar arrays were done using a different electrochemical technique than for platinum ones. Cyclic voltammetry was used in order to enhance the precision on pillar height. Actually, to reduce pillar size dispersion and improve pore filling rate, literature has proven that CV is a better choice for gold nanopillar synthesis [4]. In this work, we analyzed gold nanopillar arrays (NA) with fixed pore density (10^9 cm^{-2}) but with variable pillar geometrical parameters (height, diameter¹). As mentioned in the materials and methods section, we studied two synthesis conditions, leading to two different pillar heights, h_{15} and h_{45} (corresponding the number of the cycles for their synthesis).

Characterization of flat gold and gold nanopillar surfaces was performed by scanning electron microscopy. SEM images were further examined using an image analysis software to confirm the dimensions of nanopillars. Figure 3.5 presents an upper view of nanopillar arrays. On this figure, we can see that distribution of pillar is random and homogeneous on a large scale. Looking at a higher magnification, we can notice that the density is such that pillars are close to each other and often even merge.

We determined the 3D geometrical parameters from SEM pictures of tilted samples at an

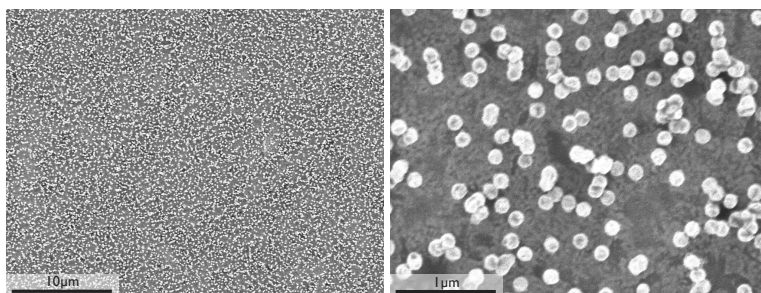


Figure 3.5: SEM pictures of self-standing gold nanopillars on a gold surface ($d = 10^9 \text{ cm}^{-2}$, $\Phi_p \sim 100 \text{ nm}$)

angle of 30° to the vertical. Table 3.1 shows the geometrical parameters obtained from the measurements of a representative number of pillars (see materials and methods section). First, we can point out that the diameter of pillar is bigger than the one announced by the supplier, but is really homogeneous on a local (one sample) or global scale (between samples). Secondly, the height of pillars is also quite uniform for both types of nanostructured electrodes. While increasing the number of CV cycles for synthesis, the height is increased, but the factor is not directly proportional. Finally, based on the measured geometrical parameters, the additional electrode surface brought by pillars (in terms of plane electrode surface) was calculated by a

¹The announced diameter is the same for all samples (100nm), but was measured to obtain the exact value.

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simple formula :

$$S_p = \pi \cdot \Phi_p \cdot h_p \cdot d \quad (3.5)$$

where S_p is the surface increase created by pillar per plane electrode surface unit ($cm^2_{pillar} / cm^2_{electrode}$). Pillar surface is shown to be significant even for small sizes of pillars. The surface increase factor is thus defined as the ratio of the total surface ($S + S_p$) versus the planar electrode surface (S). Figures 3.6 show the morphology of the synthesized nanostructured electrodes

Table 3.1: Table of structural features for gold nanopillar surfaces. Pillar heights and diameters were measured by SEM, while other dimensions were determined by the template used for surface elaboration.

Sample	density of pillars (cm^{-2})	average spacing between pillars (nm)	diameter of pillars (nm)	height of pillars (nm)	Surface increase factor
h_{15}	10^9	~ 316	127 ± 4	226 ± 15	1.9
h_{45}	10^9	~ 316	128 ± 4	482 ± 41	2.94

for the two heights. On both, we can observe the roughness of the bottom gold surface. It is attributed to the poor adhesion of polycarbonate membranes on the gold surface, which lead to the infiltration of synthesis solution inbetween, and its deposition during synthesis. As it is not possible to control this phenomenon (besides improving the nanoporous membranes fabrication process), this could be one source for an eventual deviation of proportionality between pillar height and CV cycles.

These pictures moreover confirm the possibility of merging between adjoining pillars². It is relevant at this point to mention the influence of several parameters on pillar dimensions. Namely, the importance of the concentration of the solution, the scan rate of potential for synthesis and the number of cycle will be briefly evoked. Firstly, a nearly quantitative relation between the number of cycles and the height of pillars has been shown. As for concentration of the synthesis solution, the more the dilution, the less material deposited per cycles, all other things being equal. Generally, it is better to increase the number of cycles with a diluted solution (than doing a small number of cycles with a high concentration), in order to have a better control on dimensions and well-shaped pillars. However, experiments have shown that the filling rate (the proportion of wires compared to the initial density of pores) is not controllable when the concentration is too weak. The same trend is observed while increasing scan rate of potential, with a smaller wire length deposited per cycle, in a normal range (from 10 to 200mV/s).

²The probability of merging logically increases with pillar density. It is linked with the random distribution of pores in the membrane, due to the fabrication process.

3.3. Results and discussion : effect of the structuration at the nanoscale on the electrical properties of a metallic electrode

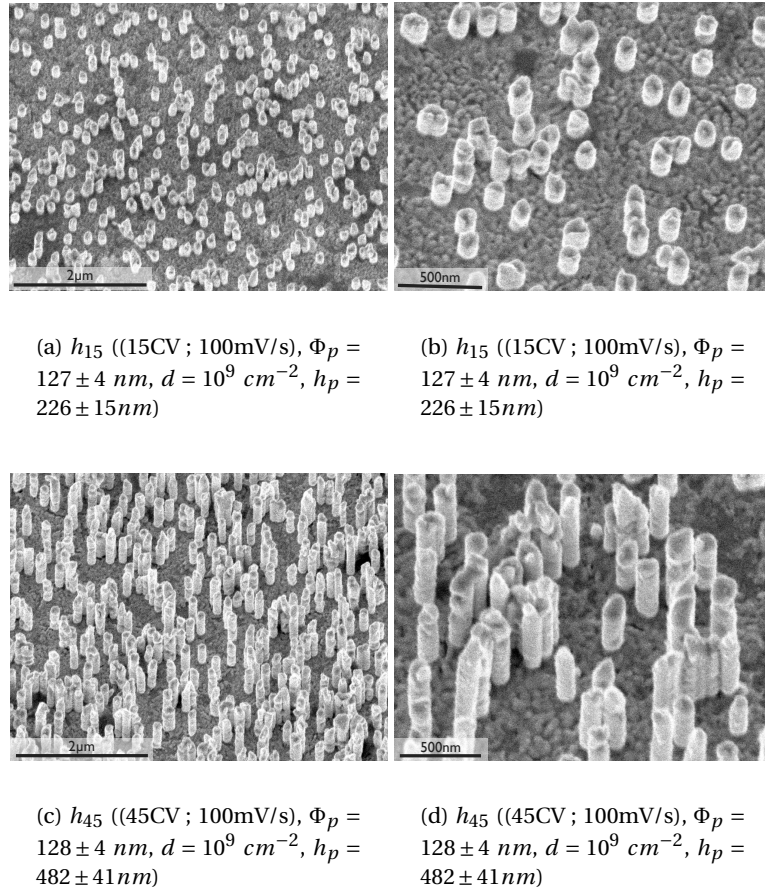


Figure 3.6: SEM pictures of h_{15} and h_{45} self-standing gold nanopillar electrodes on a gold surface.

3.3.2.2 Surface analysis by electrochemistry and electrochemical properties

The calculation of double-layer capacitances (C_{dl}) related to the synthesized electrodes were determined by cyclic voltammetry (in 0.1M NaCl at 0 mV *vs* Ag/AgCl). The link between the measured currents and double layer capacitance is done via the following equation :

$$C_{dl} = \Delta I / 2v \quad (3.6)$$

where ΔI is the current difference between positive and negative direction in CV cycle, and v the scan rate.

The values for ΔI were deduced from measurements of current during cyclic voltammetry, as shown in figure 3.7. We can observe a higher current on nanostructured electrodes and a dependance on pillar dimensions. This is attributed to the enhancement of active surface compared to flat gold, which is consistent with previous studies on nanostructured electrodes

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[4–6]. Table 3.2 gathers the surface increase factors based on double layer capacitance calcu-

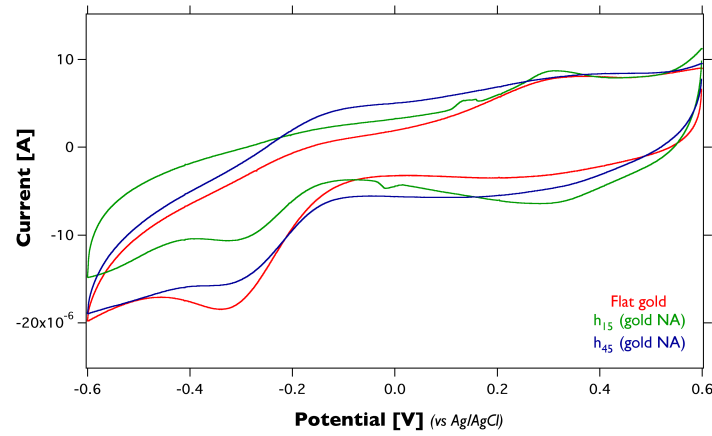


Figure 3.7: Cyclic voltammograms recorded in 0.1 M NaCl at a scan rate of 100 mV/s on flat gold (—) and nanopillar arrays (h_{15} and h_{45}).

lations, for two values of scan rate. We can firstly conclude from these factor calculations that the determination of double layer capacitance is independent of the scan rate used (for both pillar heights). In addition, there is a magnification of the C_{dl} induced by the presence of a nanostructure, and more interestingly a correlation with the size of the pillars.

Table 3.2: Table for the double layer capacity (C_{dl}) determination in 0.1 M NaCl of flat gold surface and nanopillar arrays (h_{15} and h_{45}).

Sample	ΔI [A] in NaCl 0.1M (at 0mV vs ref.)		C_{dl} [F] in NaCl 0.1M		Surface increase factor	
	10mV/s	100mV/s	10mV/s	100mV/s	10mV/s	100mV/s
Au	$0.82 \cdot 10^{-6} \pm 1.05 \cdot 10^{-7}$	$5.67 \cdot 10^{-6} \pm 0.68 \cdot 10^{-6}$	$4.12 \cdot 10^{-8} \pm 0.53 \cdot 10^{-8}$	$2.83 \cdot 10^{-8} \pm 0.34 \cdot 10^{-8}$	-	-
h_{15}	$1.25 \cdot 10^{-6} \pm 5.05 \cdot 10^{-7}$	$8.71 \cdot 10^{-6} \pm 3.20 \cdot 10^{-6}$	$6.24 \cdot 10^{-8} \pm 2.52 \cdot 10^{-8}$	$4.36 \cdot 10^{-8} \pm 1.59 \cdot 10^{-8}$	1.51	1.54
h_{45}	$1.81 \cdot 10^{-6} \pm 3.37 \cdot 10^{-7}$	$12.0 \cdot 10^{-6} \pm 1.18 \cdot 10^{-6}$	$9.06 \cdot 10^{-8} \pm 1.69 \cdot 10^{-8}$	$5.99 \cdot 10^{-8} \pm 0.59 \cdot 10^{-8}$	2.19	2.12

As previously shown, the double layer capacitance evaluation obtained from CV measurements is in agreement with the SEM-observed increase of surface. Nevertheless, even if qualitatively in the same trend, a quantitative analysis seems to indicate that the electroactive area is not as high as the one from microscopy. This discrepancy in the estimation of C_{dl} can be explained by the complex capacitance behaviour compared to regular capacitor, where this property is independent of the applied potential [6].

To overcome this limitation, and because the capacitance is a property of electrode/electrolyte interface, electrochemical impedance spectroscopy was used. This consists in the comparison of the current response to the (small) alternating potential input for the electrochemical circuit

3.3. Results and discussion : effect of the structuration at the nanoscale on the electrical properties of a metallic electrode

composed of the working electrode, the electrolyte solution and the counter-electrode. The impedance module and phase of flat and NA gold electrodes as a function of frequency in a range from 10^5 to 1 Hz are reported on figure 3.8. The aspect of the curve is typical of a metallic electrode with $|Z|$ being dominated by the uncompensated resistance of the electrolyte. This resistance is (almost) the same in all cases (differences can be attributed to a small shift in electrode distance or placement). At low frequencies, experiments exhibit a lower $|Z|$ for nano-featured electrodes. Here again, the bigger the pillar height, the lower the impedance module. As it has been explained by Cui et al. [8]: *since the electrode material and electrolyte solution are the same across all samples [...] it is the surface morphology that plays the most important role in causing the difference in impedance spectroscopy.*

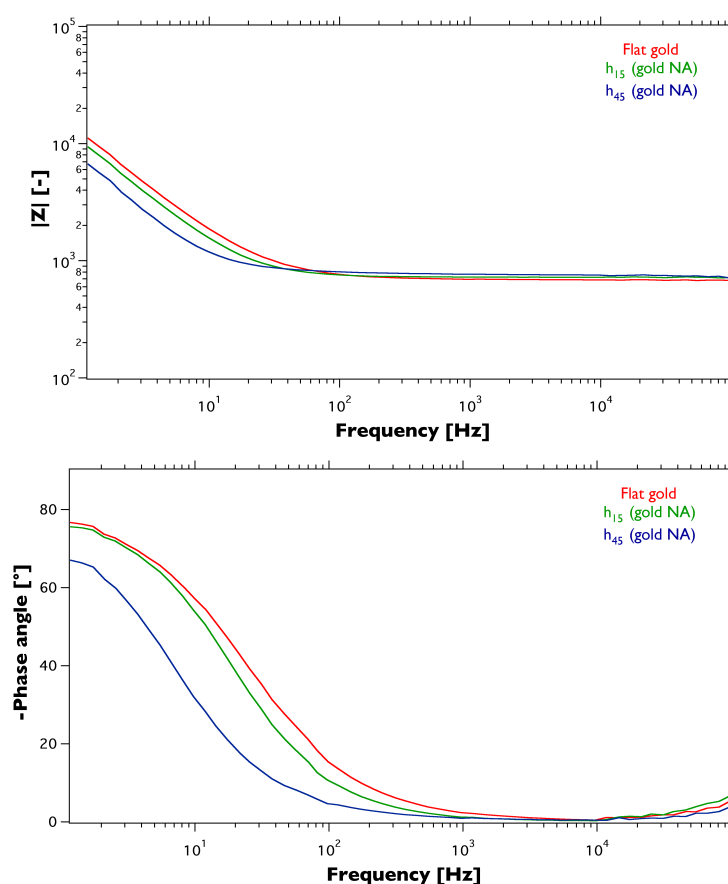


Figure 3.8: EIS Bode spectrum for the impedance modulus and phase measured in 0.1 M NaCl on flat gold (—) and nanopillar arrays (—, h_{15} and h_{45}).

To further investigate the electrochemical electrode/electrolyte properties at the electrode/electrolyte interface, data were fitted with model circuits, as defined in the materials and methods section. Table 3.3 summarizes the parameters for EIS fitting with the described model circuit in

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two different electrolytes. From these parameters, we can see at first that the solution resistance value is nearly independent of the samples. The small difference obtained for h_{45} samples could be explained by a small shift in the placement of the electrodes. The distance between working and counter-electrodes can be a part in it. As we look at the value of Q_0 we can see a trend for the microstructure. By definition, the capacitance is proportional to the active area. As $Q_0 = 1/|Z|$ at $\omega=1\text{rad/s}$, it is used as an approximation of the capacitance with values of n close to 1, as it has already been described in literature [8]. Here, the n value is approximately 0.9. While comparing Q_0 values, we observe that this is higher with a nanostructure compared to gold, and even higher with nanostructure of bigger dimensions. The variance of calculations is large compared to the values of admittance. We will thus be careful in the quantitative interpretations. We can nevertheless notice the same trend as reported in literature, for both electrolytes. Compared to SEM-measured surface increase factors, the values obtained here are lower. Schröper et al. observed the same discrepancy between surface increase from EIS and SEM, but in the opposite direction. They did not address the problem further on. No answer can be found in the literature about this discrepancy between SEM and EIS measurements. One hypothesis could be that the whole active surface may not be free of some residual polycarbonate. Even if this does not seem the case in the observations done by SEM and that the dissolution process was optimized in order to reveal the entire surface of both pillars and gold background surface, there can remain resistive residues of PC on the surface of certain pillars, leading to a decrease in surface active area compared to microscopic observations.

Table 3.3: Table of fitted EIS parameters for flat and NA gold electrodes (h_{15} and h_{45}). Equivalent circuit for fitting is a $R-CPE$ serial circuit model for all types of structure. Parameters are presented for 2 types of electrolytes ($NaCl$ 0.1M and $K_4Fe(CN)_6$ 5mM + KCl 0.1M)

Sample	EIS in NaCl 0.1M (at 0.6V vs ref.)		
	R [Ω]	CPE (Q_0) [$S.s^{1/2}$]	CPE (n) [-]
Au	848 ± 245	$1.44 \cdot 10^{-5} \pm 0.27 \cdot 10^{-5}$	0.893 ± 0.04
h_{15}	818 ± 188	$1.73 \cdot 10^{-5} \pm 1.28 \cdot 10^{-5}$	0.869 ± 0.04
h_{45}	1112 ± 184	$2.41 \cdot 10^{-5} \pm 1.15 \cdot 10^{-5}$	0.863 ± 0.03

Sample	EIS in $K_4Fe(CN)_6$ 5mM (at 0.6V vs ref.)		
	R [Ω]	CPE (Q_0) [$S.s^{1/2}$]	CPE (n) [-]
Au	662 ± 205	$1.61 \cdot 10^{-5} \pm 0.25 \cdot 10^{-5}$	0.893 ± 0.04
h_{15}	687 ± 188	$1.87 \cdot 10^{-5} \pm 0.86 \cdot 10^{-5}$	0.865 ± 0.05
h_{45}	839 ± 139	$2.90 \cdot 10^{-5} \pm 0.27 \cdot 10^{-5}$	0.874 ± 0.03

	Surface increase factor	
	100 mV/s	10 mV/s
Au	-	-
h_{15}	1.20	1.16
h_{45}	1.67	1.80

From the above results, we can conclude that there is an improvement on impedance of the electrodes brought by the nanostructuration, even if the quantitative correlation is not direct

3.3. Results and discussion : effect of the structuration at the nanoscale on the electrical properties of a metallic electrode

and require more investigation. In order to have another look at the charging properties of the solid/liquid interface, we used a well-known redox couple as a diffusion-controlled system to investigate further on [4–6]. Figure 3.9 shows representative CV curves for plane and nano-featured electrodes in a 5mM $K_4Fe(CN)_6$ + 0.1M KCl electrolyte, at two different scan rates. For the $(Fe(CN)_6)^{4-}/3-$ system, the faradaic redox transfer (Fe^{2+}/Fe^{3+}) shows marked cathodic and anodic waves. Peak potentials are recorded at +0.4(0.3) V for oxidation and 0.03(0.12) V for reduction at 100 (10) mV/s, at the same potentials for flat gold and nanostructures.

The measured current peaks are summarized in table 3.4. Confirming the observation on the

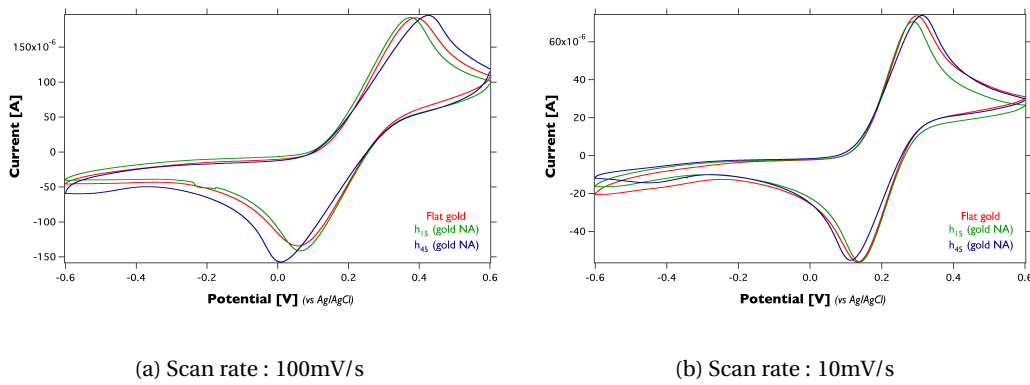


Figure 3.9: Cyclic voltammetry of on flat gold (red) and gold nanopillar arrays (h_{15} and h_{45}) electrodes in 5mM $K_4Fe(CN)_6$ with 0.1M KCl as a supporting electrolyte at two different scan rates.

previous graphs (figure 3.9), the current peaks display the same values. The active surface in the redox process thus corresponds to the geometric surface of the electrode at the relatively low scan rates used in these experiments.

Table 3.4: Table of redox currents determination in 5mM $K_4Fe(CN)_6$ + 0.1M KCl for flat gold surface and nanopillar arrays (h_{15} and h_{45}) at two scan rates.

Sample	CV in $K_4Fe(CN)_6$ 5mM ([−0.6 ; 0.6]V vs ref. ; 100mV/s)				
	I_{ox} [A]	E_{ox} [V]	I_{red} [A]	E_{red} [V]	ΔI [-]
Au	$1.72 \cdot 10^{-4} \pm 0.39 \cdot 10^{-4}$	0.41 ± 0.04	$-1.43 \cdot 10^{-4} \pm 0.16 \cdot 10^{-4}$	0.03 ± 0.02	$3.15 \cdot 10^{-4} \pm 0.45 \cdot 10^{-4}$
h_{15}	$1.79 \cdot 10^{-4} \pm 0.51 \cdot 10^{-4}$	0.40 ± 0.02	$-1.38 \cdot 10^{-4} \pm 0.29 \cdot 10^{-4}$	0.04 ± 0.02	$3.18 \cdot 10^{-4} \pm 0.79 \cdot 10^{-4}$
h_{45}	$1.82 \cdot 10^{-4} \pm 0.07 \cdot 10^{-4}$	0.43 ± 0.01	$-1.51 \cdot 10^{-4} \pm 0.01 \cdot 10^{-4}$	0.05 ± 0.06	$3.26 \cdot 10^{-4} \pm 0.12 \cdot 10^{-4}$

Sample	CV in $K_4Fe(CN)_6$ 5mM ([−0.6 ; 0.6]V vs ref. ; 10mV/s)				
	I_{ox} [A]	E_{ox} [V]	I_{red} [A]	E_{red} [V]	ΔI [-]
Au	$7.18 \cdot 10^{-5} \pm 0.72 \cdot 10^{-5}$	0.31 ± 0.02	$-6.13 \cdot 10^{-5} \pm 0.74 \cdot 10^{-5}$	0.12 ± 0.01	$1.33 \cdot 10^{-4} \pm 0.11 \cdot 10^{-4}$
h_{15}	$7.37 \cdot 10^{-5} \pm 1.68 \cdot 10^{-5}$	0.30 ± 0.01	$-6.01 \cdot 10^{-5} \pm 1.00 \cdot 10^{-5}$	0.12 ± 0.01	$1.34 \cdot 10^{-4} \pm 0.26 \cdot 10^{-4}$
h_{45}	$6.35 \cdot 10^{-5} \pm 0.28 \cdot 10^{-5}$	0.31 ± 0.01	$-6.04 \cdot 10^{-5} \pm 0.33 \cdot 10^{-5}$	0.11 ± 0.01	$1.27 \cdot 10^{-4} \pm 0.07 \cdot 10^{-4}$

	Surface increase factor	
	100 mV/s	10 mV/s
Au	-	-
h_{15}	1.01	1.01
h_{45}	1.03	0.95

3.4 Results and discussion : influence of a model nanostructuration on osteoblasts cells behaviour

3.4.1 Characterization of gold nanowire arrays for cell culture

Characterization of flat gold and gold nanopillar surfaces was performed by scanning electron microscopy. SEM images were further examined using an image analysis software to confirm the dimensions of nanopillars. At this scale, the bottom gold surface can be considered as perfectly plane, even though a small roughness may be observed at a higher scale. Morphologies of surfaces with nanopillars synthesized using a simple hard-templating electrochemical method can be observed on figures 3.10 for low and high densities. The structural features of the obtained gold nanostructured surfaces are summarized in Table 3.5. Pillar density and diameter, as well as interpillar spacing, are deduced from the template characteristics, while their height was measured from SEM images.

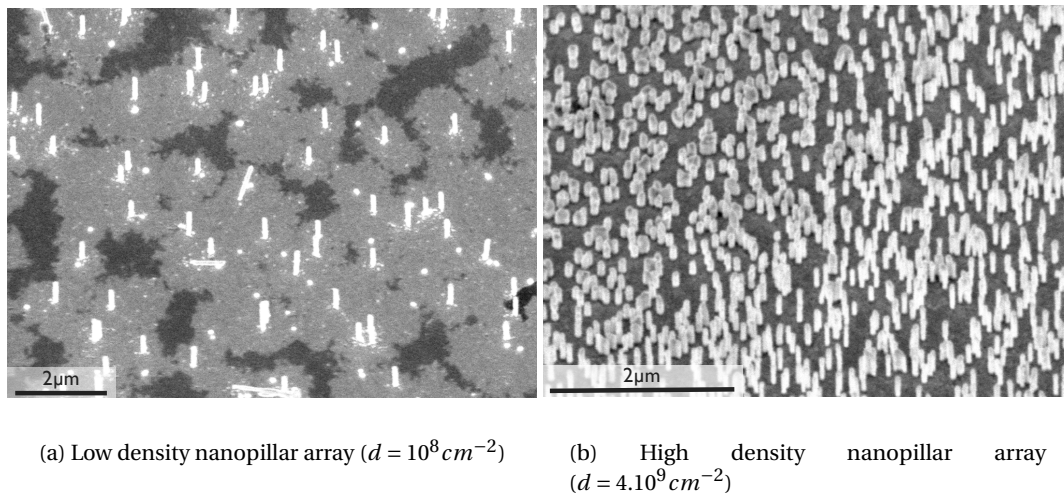


Figure 3.10: SEM images of low (a) and high (b) densities gold nanopillar arrays at different scales.

Sample	density of pillars (cm^{-2})	diameter of pillars (nm)	height of pillars (nm)	average spacing between pillars (nm)
Low density	10^8	100	287 ± 81	1000
High density	4.10^9	100	219 ± 76	160

Table 3.5: Structural features of low and high density gold nanopillar surfaces. Pillar height was measured by SEM, while other dimensions were determined by the template used for surface elaboration. Template characteristics were provided by the manufacturer.

3.4. Results and discussion : influence of a model nanostructuration on osteoblasts cells behaviour

3.4.2 Effect of the nanotopography on cell morphology using SEM

SEM images of figure 3.11 show the morphology of cells on flat gold and on gold nanopillars after 72h in culture. Cell morphology is significantly affected by the presence of nanopillars, whatever their density. A spread and polygonal morphology is indeed observed on flat gold, while cells become significantly elongated on gold nanopillars with low and high densities. This difference in cell morphology actually appears at an earlier stage of cell culture. This will be discussed in terms of focal contact formation and actin organization in the following paragraphs.

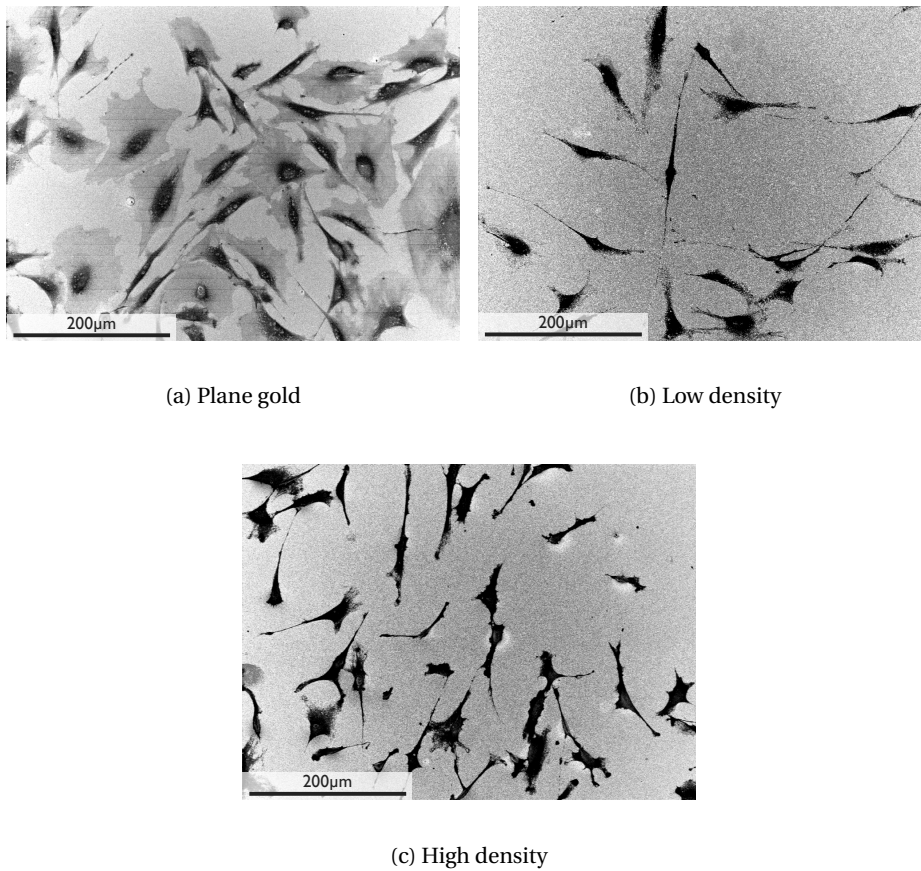


Figure 3.11: MC3T3 osteoblast cells morphology analyzed by SEM after 72 hours of cell culture on different substrates.

Quantifications of the observed changes in cell morphology, in terms of mean cell area and cell circularity, after 72h of culture on flat and nanopillar samples are reported in figure 3.12. The mean cell area for cells grown on flat gold surface is significantly higher compared to those of low density ($p < 0.01$) and high density ($p < 0.01$) nanopillars. However, there is no significant difference between mean cell areas on the surfaces with two different densities of nanopillars

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(figure 3.12.A). Cell shape was analyzed through circularity measurements, as explained in materials and methods section. On flat gold surface, cells were polygonal and spread, resulting in a high circularity. On both low density ($p < 0.001$), and high density ($p < 0.001$) nanopillars, cell circularity was significantly lower compared to flat gold surface, as a result of their more elongated structure. Again, no significant difference in circularity was observed between two different nanopillar densities.

It is clear that these changes in cell morphology are caused by the topographical features. The

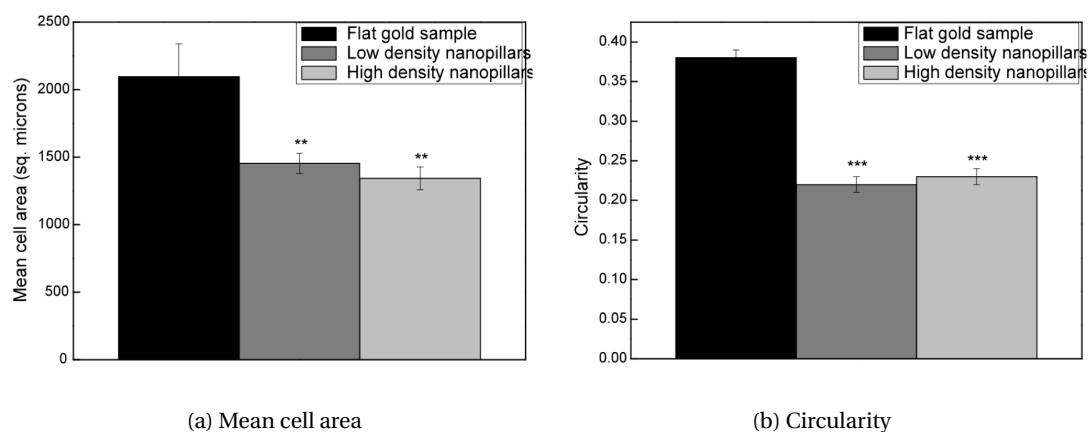


Figure 3.12: Quantification of cell morphology, achieved from SEM images, in terms of mean cell area and circularity of MC3T3 E1 pre-osteoblast cells after 72h of culture on flat gold and nanopillars at low and high density. (Error bars are standard error from the mean where $n = 50$).

previous work of Sjöström et al. on the effect of different heights of titanium nanopillars on MG63 osteoblast cells endorses these observations [38, 39]. However, Sjöström et al. used pillars of varying diameters, height and spacing, and thus the exact correlation between cell behavior and nanotopographical parameters cannot be generalized. Nevertheless, their work suggests that when the spacing between pillars is higher than 40 nm, human MG63 osteoblast cells acquired an elongated morphology. Especially, significant reductions in cell attachment and elongation were observed for pillars with 100 nm height and 115 nm spacing compared to lower pillar height and smaller spacing [39]. This is consistent with our observations, where spacing between nanopillars was ~ 160 nm for high and $\sim 1\mu\text{m}$ for low density nanopillar samples, on which cells were predominantly elongated.

Even though cell morphology was not affected by the density or height of nanopillars, as shown from mean cell area and circularity, differences in cell protrusions formation were observed between different densities of nanopillar samples. When a cell adheres to a substrate, it scans its surrounding environment using protrusions called filopodia and lamellipodia. Filopodia help cells to attach and move on substrate. These structures are of nanometric scale (250-400 nm) and are thus likely to be influenced by the underlying nanotopography [40]. It is believed that these

3.4. Results and discussion : influence of a model nanostructuration on osteoblasts cells behaviour

changes in filopodia interactions with nanotopography result in changes in cell morphology [18, 41].

Figure 3.13 illustrates the cell interactions with both flat gold and gold nanopillar surfaces, based on SEM images of cells after 4h of culture. Figures *a*, *d* and *g* show cell morphology, figures *b*, *e* and *h* show cell interactions to the different surface topographies and figures *c*, *f* and *i* show the size and shape of cell protrusions on flat gold, low and high density nanopillar surfaces, respectively.

Cells grown on flat gold acquire a more polygonal and spread morphology compared to

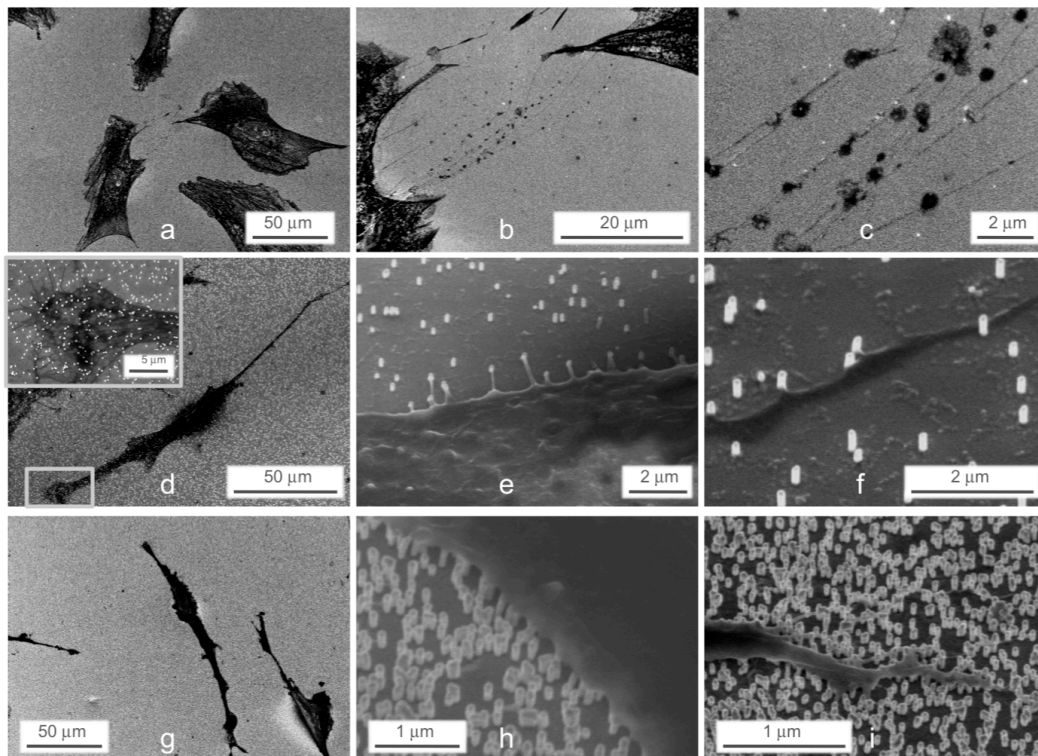


Figure 3.13: SEM pictures showing the interactions of MC3T3 pre-osteoblast cells after 4h of culture on flat gold (*a-c*), low density (*d-f*) and high density (*g-i*) nanopillars. High magnification images show cell interaction with nanotopography and filopodia formation.

both nanopillar surfaces (and maintain such phenotype even in longer culture time, this will be discussed later) In response to a flat gold surface, cells produce very thin protrusions of nanometer-scale diameter (as shown in Figures 3.13.b and 3.13.c). Cells also deposit small anchoring points, seen as submicron dots as shown in Figure 3.13.c, which they use as guide for the deployment of their protrusions on this surface. On low density nanopillars, cells support their attachment by clinging onto the pillars via short microvilli (figure 3.13.e). On this surface, cell protrusions tend to use pillar bases as anchoring points and remain relatively thin. The images of cells on high density nanopillars (figure 3.13.h) show that the cell membrane is

Chapter 3. Effect of a model structuration at the nanoscale on a metallic electrode on the electrical properties and osteoblast cell behaviour

supported on a bed of nanopillars (*ie* fakir cells). On these samples, cells produce thicker cell protrusions compared to flat gold and low density nanopillar samples. A trend seems to emerge between density of nanopillars, and size and shape of cell protrusions, where cell protrusions become longer and thicker from flat gold to increasing density of nanopillars. This is believed to be linked to cell mobility on these surfaces, as will be discussed later.

3.4.3 Effect of the nanotopography on cytoskeletal organization of cells

High magnification fluorescence microscopy images of cells after 4h of culture on flat gold surface begin to show some organization of actin stress fibers, which are absent in the case of cells on high density nanopillars (figure 3.14). Cytoskeleton arrangement is indeed significantly affected by the presence of nanopillars, where actin remains in the cortical axis of cells and no actin stress fiber formation is observed. After 24 h, actin organization and stress fiber formation become more pronounced on flat gold, while cells acquire an elongated morphology on nanopillars. Vinculin staining on flat surface shows a clear localization at the focal contacts of cells. However, such localization is not observed on nanopillars. The number of focal adhesion points, as revealed by vinculin staining, increases on flat gold, but not on nanopillars. This trend continues even after 72h of culture, on both density nanopillars as further illustrated in figure 3.15, which shows higher magnification images.

It is believed that focal adhesion formation, which consists of protein clustering at a micron scale, is either not possible or their formation becomes slower due to the topography created by nanopillars. Lutz et al. showed that by restricting cell-matrix adhesion area to $< 1\mu m^2$, cell morphology, actin dynamics, migration and ECM assembly of cells are affected [42]. In their work, they used patterns of RGD peptides with variable dimensions ranging from 250 to 2000nm. They showed that when the pattern area is $< 1\mu m^2$, cells only form focal complexes, while on larger patterns cells were able to convert these complexes into mature focal contacts. Their work also suggests the importance of maturation of focal complexes for effective actin stress fibre formation, cell spreading and directional polarization. In the present work, nanopillars of 100 nm diameter provide $< 1\mu m^2$ pattern areas.

Thus, it is likely that even though cells can form focal complexes on the top of these nanopillars, their maturation to focal adhesions is not possible. This further leads to a lack of stress fibre formation which affects cell shape. Evidence of this phenomenon can be seen as early as 4h with cells grown on nanopillars (see figure 3.14). Actin only assembles around the cells cortex, without actin stress fibers. This appears to be resulting from a non-maturation of focal complexes to focal adhesions, which is in agreement with work of Lutz et al. [42].

3.4. Results and discussion : influence of a model nanostructuration on osteoblasts cells behaviour

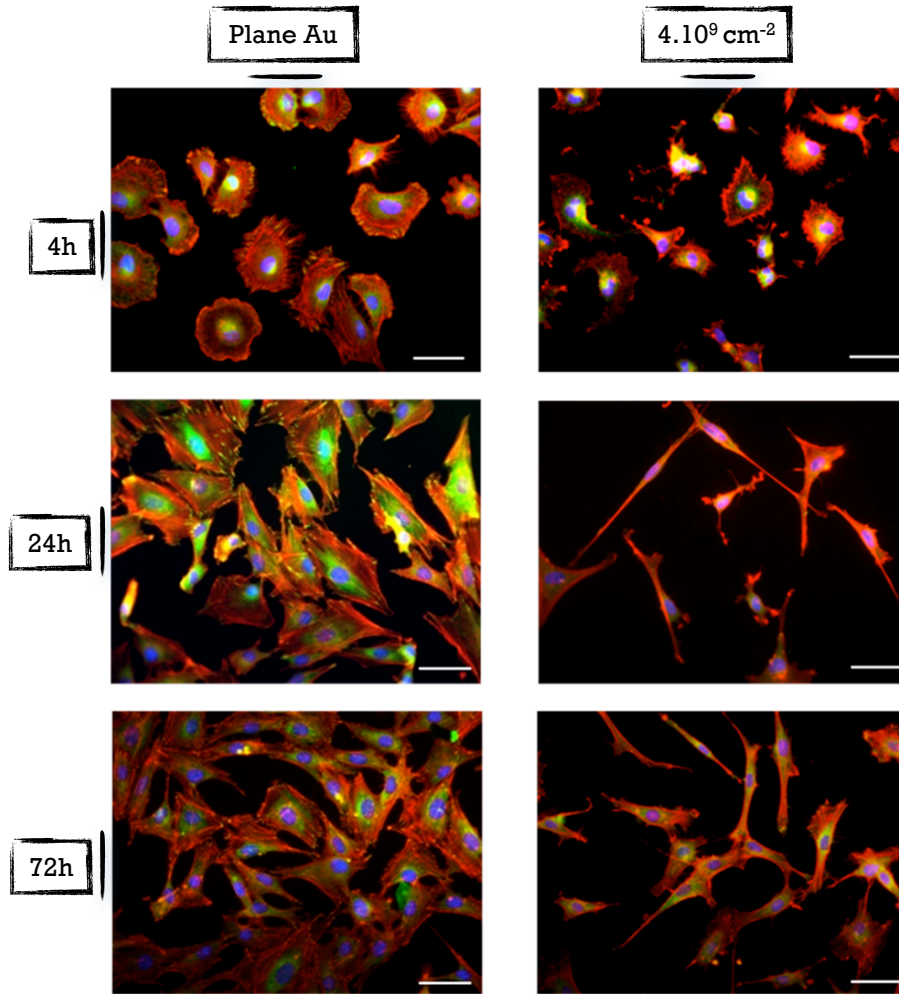


Figure 3.14: MC3T3 pre-osteoblast cells morphology on flat gold and high density gold nanopillars, after different times in culture (top: 4h, middle: 24h, bottom: 72h). Scale bar 50 μ m. F-actin is stained in red, vinculin in green and nucleus in blue.

3.4.4 Effect of gold nanopillars on osteoblast cell growth probed by Alamar blue assay

The metabolic activity of cells on the studied surfaces was analyzed using Alamar blue assay (figure 3.16.a). On flat gold surfaces, we observe a roughly linear increase in the cell metabolic rate over the period of culture. When cells were cultured on gold nanopillars, cell activity was significantly lower compared to flat gold, and was found to vary depending upon density of nanopillars. Cells on high density nanopillars show an increasing metabolic activity over the period of culture, however, it is significantly lower ($p < 0.01$ at 72h) than on low density nanopillar. Since Alamar blue is a metabolic assay, the actual cell number on these surfaces was confirmed by the counting of *DAPI* stained nuclei after 72h of culture (shown in 3.16.b). In agreement with

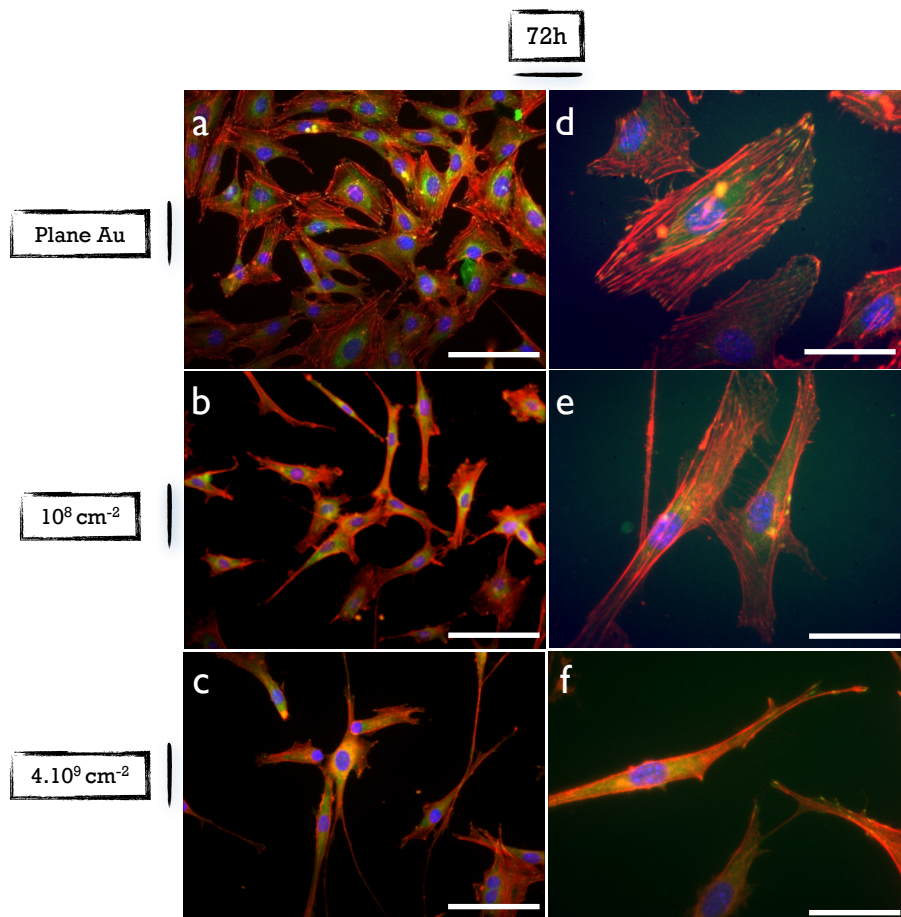


Figure 3.15: Fluorescence microscopy images of immunostained MC3T3 pre-osteoblast cells after 72h in culture on flat gold (a, d), low density gold nanopillar (b, e) and high density nanopillars (c, f). Images a,b,c are low magnification images with scale bar $50\mu m$, whereas d,e and f show high magnification images with scale bar $25\mu m$.

the Alamar blue assay results, counting of adherent cells suggests that the cell number on both nanopillar densities is significantly lower ($p < 0.001$) compared to flat gold. In addition, there is no significant difference ($p = 0.86$) in the cell number between the two different densities of nanopillars. This suggests that even though the number of adherent cells is not significantly affected by the nanopillar density, metabolic activity on nanostructured surfaces is lower when a high density of nanopillars is used compared to a lower one. In the literature, topographical cues with different shapes are shown to have significant effects on various cell functions such as cell adhesion [43–45], morphology (orientation, spreading and polarization) [44, 46–48], focal adhesion formation [46], proliferation [45, 46, 49], differentiation [24, 43] and gene expression [47, 50, 51]. Changes in cell number and metabolic rate observed in this work on nanopillars compared to flat gold are thus in agreement with previous works where such link between

3.4. Results and discussion : influence of a model nanostructuration on osteoblasts cells behaviour

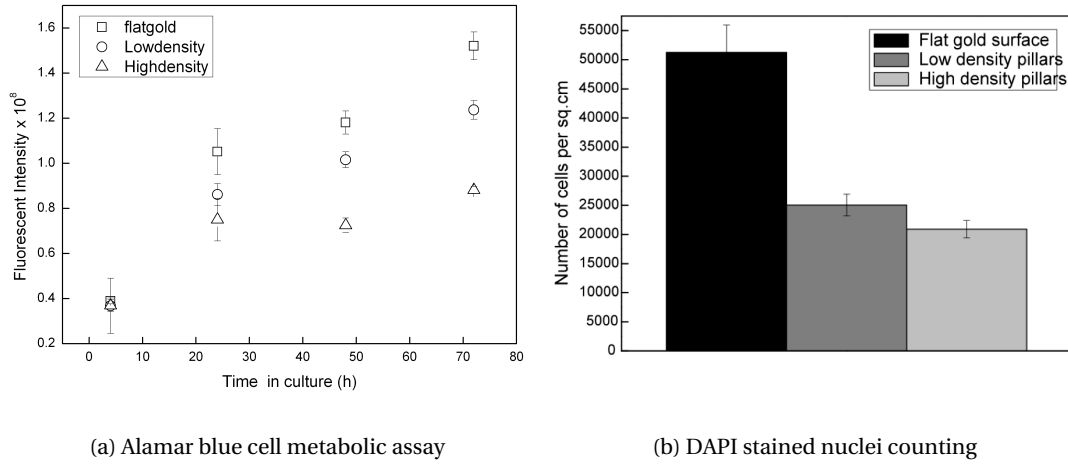


Figure 3.16: Cell activity as measured by using Alamar blue cell metabolic assay over a period of culture of 72h, and cell counting using DAPI stained nuclei after 72h of cell culture. Error bars are standard error from the mean, with n=3.

changes in morphology and cell functions has been established. Elongation and stretching of cells were shown to induce deformations of the nucleus as compared with spread morphology of cells cultured on flat surfaces [52, 53]. These deformations of the nucleus directly influence the cell behavior through the displacement of genes inside the nuclear matrix or indirectly by signal transduction [18, 54].

However, the observed difference in the metabolic activity between the two nanopillar densities, which however lead to similar cell morphologies, is still unexplained. One possible answer lies in the complex setup of the cellular functions. Such functions are believed to be linked to the way cell protrusions are formed on these surfaces, as shown in 3.13. On flat surfaces, cell protrusions are small and short. However, with the presence of an increasing number of nanopillars, the length and size of these protrusions increase. As the dimensions of these protrusions can be linked to the mobility of cells, this latter is believed to be much more restricted on high density compared to low density nanopillar surfaces. Cell protrusions should thus retract faster due to the smaller number of pillars on low density compared to high density samples. On flat gold, cell mobility would be higher compared to both densities of nanopillars, as reflected by the higher metabolic activity.

3.5 Conclusion

In this chapter, we described the synthesis technique and parameters for the preparation of nanopillar arrays of different materials. The possibility to control the final structure by varying the synthesis parameters was demonstrated. As confirmed by electronic microscopy observations, the increase of active surface area is significant and can be enhanced by the controlled increase of pillar height. Moreover, the structure is well defined and possess peculiar properties in the scope of stimulation electrodes. As confirmed by different electrochemical characterization methods, the nano-featured surfaces show an improvement in impedance and current conductivity. Still, there are questions that need to be addressed, especially concerning the diffusion-controlled electrochemical properties. In this perspective, the effects of pillar density on diffusion-controlled electrochemical systems in particular (and electrical properties in general) need to be determined.

Looking forward to the aim of this thesis, the creation of nanopillar arrays without altering the chemical properties of the material allows the further modification of this electrode (either chemical, mechanical, biological...). Furthermore, beside stimulation properties, the structure by itself is presumed to have distinctive effects in tissue interaction.

In a second time, we studied the effects of controlled nanotopography (*ie* uniform chemistry) on preosteoblast cells growth, using both metabolic assays and microscopy observations. The nanotopography was elaborated through a simple electrochemical technique to create nanopillars of controlled dimensions (diameter, height and density). It was first shown that the presence of gold nanopillars on the surface significantly affects cell adhesion, morphology and metabolic activity. On nanostructured surfaces, the cells adopt an elongated morphology and their metabolic activity is reduced. Cell filopodia formation also showed a unique response to the created nanotopographies, and a link could be observed between the density of nanopillars at the interface and the dimensions of cell protrusions.

Interactions between cells and topography could be tailored as an alternative signaling mechanism, in the sense of tuning cell functions (ex. cell death, attachment, genetic manipulation, adhesion, migration, polarization...). This study thus contributes to the understanding of cell response to nanoscale cues of well-defined dimensions. It is also a promising approach for preparing biomaterials with well-defined nanotopography for controlling and guiding cell behaviours.

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4 Dexamethasone electrically controlled release from polypyrrole-coated electrodes

This chapter reports the systematic analysis of the drug delivery from a polypyrrole film synthesized on different electrode surfaces. The fabrication of nanostructured electrodes covered with a polypyrrole films containing anti-inflammatory molecules with controlled characteristics is studied. The drug delivery by cyclic voltammetry is then analyzed, with the effect of technique parameters. The limits of the use of this technique are pointed out.

In the second part, electrode characteristics are optimized and a more in-vivo oriented technique is studied for the controlled release of anti-inflammatory: potential biphasic pulses stimulation. The influences of technique parameters on the release characteristics are studied, with regard to in-vivo restrictions. Finally, the first results of local in-vivo liberation of anti-inflammatory molecules are shown on a rat model.

The first part of this chapter is the subject of a published paper in the Journal of Materials Science: Materials in Medicine [1]. The device for the biphasic potential pulse stimulation is the object of a patent [2].

4.1 Introduction

One of the biomaterials strategy currently explored to reduce or eliminate the undesired early inflammatory tissue reaction and maintain signal transmission quality over time is the delivery of anti-inflammatory molecules in the vicinity of the implant. In fact, many studies have brought into light the advantages of a local and controlled delivery of molecules in the direct environment of a stimulation apparatus. In this perspective, polymers have been shown to offer many advantages in the fabrication of scaffolds and coatings for electrical stimulation. More precisely, conducting polymers were widely studied, because of their ability to conduct electricity on one hand, and for their aptitude to deliver molecules on the other hand [3]. Among conjugated polymers, polypyrrole (PPy) is particularly attractive because of its easy and efficient electrosynthesis from aqueous solutions, biocompatibility, good environmental stability and low voltage actuation [4]. Up to now, PPy has already been studied for controlled delivery of different biomolecules, such as adenosine 5'-triphosphate (ATP) [5], neurotrophin [6, 7] and dexamethasone [8].

Dexamethasone is a synthetic glucocorticoid drug with anti-inflammatory and immunosuppressant effects. Its activity is achieved through glucocorticoid receptors present in most neurons and glial cells throughout the brain, and involved in the inflammatory pathway and on the pro-inflammatory cytokines [9]. Dexamethasone is widely used in systemic injections, and has been shown to effectively minimize the reactive tissue response to neural implants in rat [10]. Figure 4.1 represents the chemical structures of dexamethasone (DEX) and dexamethasone phosphate disodium salt. Over the past few years, researchers have developed systems for the delivery

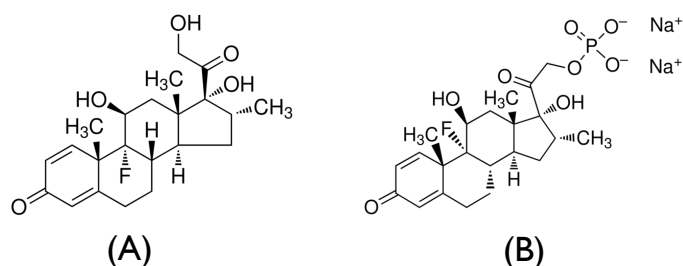


Figure 4.1: Chemical structures of dexamethasone (A) and dexamethasone phosphate disodium salt (B).

of dexamethasone in a local and site-specific perspective, in order to overcome the damage engendered by systemic large doses exposure of the entire body. Indeed, systemic injections of DEX were shown to reduce tissue reaction around the implant in the central nervous system [10, 11], but this kind of DEX administration is very expensive and can cause serious side effects, such as myopathy and diabetes [12, 13]. On the contrary, the time sustained local release of dexamethasone has previously been proved to have anti-inflammatory and anti-fibrotic effects while used in stents [14–16]. More precisely, local delivery of drugs by the implant itself is a

promising and more effective strategy. In this respect, Zhong et al. [17] developed a DEX eluting nitrocellulose-based coating on silicon neural probes that was proven to attenuate the inflammatory response and to reduce neuronal loss in the vicinity of the coated probes. However, this passive delivery system, based on simple drug diffusion, does not allow the entire control on the released drug doses.

More recently, researchers have developed new ways of delivering dexamethasone by the implant itself via a conducting polymer coating. Stevenson et al. [14, 18] synthesized polyterthiophene films doped with DEX and studied the delivery from those films by the application of oxidizing potentials through different protocols. They also observed a spontaneous release of DEX (without electrical stimulation) due to the auto-reduction of polyterthiophene when placed into saline phosphate buffered receiving solution. In fact, no statistical difference was observed between the unstimulated (auto-reduced) polymer and the electrochemically reduced polyterthiophene. More interestingly, Wadhwa et al. reported on a PPy-coated electrode which allows a local and electrical control over the amount of DEX released [8]. They studied the electrochemical deposition of dexamethasone-doped polypyrrole films on gold surfaces using a potentiostatic mode in function of the charge density applied. They proved the controlled delivery from the synthesized films by cyclic voltammetry, with a relation of the quantity delivered in function of the number of cycles applied. In vitro murine glial cell cultures were done, and the release of drug from the polymer coating was proved not to produce any toxic side effects, as confirmed by healthy neuronal growths. In fact, the released drug caused increased neurite formation when compared to control non-covered electrodes. However, several problems were highlighted, in particular chemical and mechanical instability of the polymer. They observed macroscopic cracks on the polymer surface after a few electrochemical stimulation cycles, suggesting that the film undergoes physical stresses upon swelling and shrinking cycles, leading to its delamination and breakdown. This is, of course, detrimental for the long-term performances.

In the present work, we report a study on the liberation of dexamethasone from polypyrrole coated electrodes with different characteristics. Firstly, a successful and reproducible process to elaborate PPy nanostructured electrodes for improved electrically controlled and local delivery of anti-inflammatory is designed [1, 19]. The strategy developed for preparing the nanobioelectrodes is based on the hard templating method that presents the significant advantage of leading to the production of nanotubes or nanowires with monodisperse and controlled diameters and lengths [20]. In this study, platinum was electrodeposited into the nanopores of an Au-coated polycarbonate membrane. The further dissolution of the membrane leads to the formation of a brush of vertically aligned metallic nanowires on the substrate. Then, a one step electropolymerization procedure was used to deposit PPy films doped with DEX onto the Pt nanopillar brush. By controlling the polymerization time, nanostructured electrodes including PPy/DEX films of different thicknesses were prepared. Electrochemically controlled DEX release was achieved

Chapter 4. Dexamethasone electrically controlled release from polypyrrole-coated electrodes

and it was found that the system is a true electrically controlled release system. A study of the electroactivity and morphology of the nanostructured PPy/DEX electrodes before and after drug release was carried out by cyclic voltammetry and scanning electron microscopy (SEM), respectively. It was found that the presence of metallic nanopillars onto the electrode surface improves the adherence between the polymer and the electrode and increases the electroactivity of the PPy coating. In this study, we highlighted some limits of the cyclic voltammetric used to deliver the molecules, in the outlook of in-vivo applications. There was thus a need to develop a new approach for a controlled drug delivery.

In a second time, we optimized the synthesis technique, in order to improve the reproducibility in the elaboration of the films and the delivery of dexamethasone. A double-layer system was developed, with a first layer containing no molecule of dexamethasone (but smaller ions). The presence of this layer on the metallic electrode reduced the synthesis difficulties engendered by the poor conductivity of the monomer solution containing dexamethasone, and thus improved the reproducibility in the synthesis of a second layer containing the dexamethasone molecules. Afterwards, we developed a technique for the controlled delivery of dexamethasone with respect to the limitations induced by a complex release medium and the possible occurrence of side reactions. The influences of biphasic pulse potential stimulation parameters were assessed together with the design and implementation of a device for the administration of the electrical stimulation. Finally, preliminary in-vivo implantations in a rat model were accomplished and proved the technique to be effective.

4.2 Materials and methods

4.2.1 Preparation of platinum nanowire arrays

The nanopillar brushes were prepared by an electrochemical template method. The electrochemical growth of Pt nanowires was carried out in a commercial one compartment Teflon cell *CH Instruments* at room temperature with a Pt disk counter-electrode and an Ag/AgCl reference electrode. The electrosynthesis was controlled using an *Egg Princeton Applied Research 273A* potentiostat/galvanostat. Nanoporous polycarbonate (PC) track-etched membranes with a thickness of 21 μm , pore diameter of 150 nm and a pore density of 10^8cm^{-2} were supplied by *it4ip* [21] and used as templates. A 450 nm gold layer was previously evaporated on one side of the membrane, serving as working electrode. The Pt nanowires were electrodeposited potentiostatically on disk samples (Area = 0.282cm^2) at 0V from a homemade solution of 0.01M $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ and 0.5M H_2SO_4 in de-ionized water. The amount of material deposited within the nanopores was controlled by the total electrical charge passed during the electrodeposition process. After deposition of the Pt nanowires, the PC template was removed by dissolution in dichloromethane. The brush of Pt nanopillars attached to the gold surface was finally rinsed with a 0.5 M NaOH solution in order to remove any trace of PC onto the nanowires.

4.2.2 Polypyrrole films synthesis

❶ PPy/DEX films were grown potentiostatically onto the nanostructured electrodes (see figure 4.2). The same electrochemical cell, counter and reference electrodes and electrochemical equipment than those used for the synthesis of Pt nanopillars were used. The electrosynthesis solution consisted in 0.1M Py (*Sigma-Aldrich*, purified over alumina until colorless) and 0.025M DEX (*Sigma-Aldrich*, dexamethasone 21 phosphate disodium, used as received) in Milli-Q water of 18 M Ω .cm resistivity. The films were synthesized by applying a constant potential of 0.8V and automatically stopped when the desired charge was reached. PPy/DEX films were then rinsed several times with Milli-Q water.

❷ Two-layer films were grown potentiostatically onto platinum or gold flat electrodes ((see figure 4.2). The same electrochemical cell, counter and reference electrode than for the previous synthesis were used. The electrosynthesis solutions consisted in 0.1M Py (*Sigma-Aldrich*, purified over alumina until colorless) and 0.1M NaCl (*Sigma-Aldrich*) (1st layer) and 0.025(0.05)M DEX (2nd layer) in Milli-Q water of 18 M Ω .cm resistivity. The films were synthesized by applying a constant potential of 0.8V (*vs* Ag/AgCl) and automatically stopped when the desired charges were reached (in general 50mC/cm² for PPy/Cl and 100mC/cm² for PPy/DEX). The bilayer films were then rinsed several times with Milli-Q water.

Chapter 4. Dexamethasone electrically controlled release from polypyrrole-coated electrodes

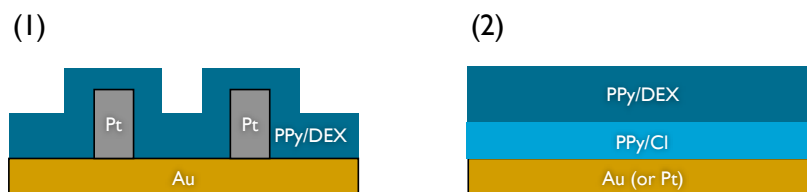


Figure 4.2: Diagram of the polypyrrole-coated samples: single PPy/DEX layer on Pt nanopillar arrays (on a gold substrate) (1) and two-layer polypyrrole film (PPy/Cl + PPy/DEX) on a flat gold (or platinum) surface (2).

③ Two-layer films were grown potentiostatically onto cuff electrodes prepared by MOEX lab - UCL (see figure 4.3 for cuff general scheme). The same electrochemical cell, counter and reference electrode than for the previous synthesis were used. The electrosynthesis solutions consisted in 0.1M Py (*Sigma-Aldrich*, purified over alumina until colorless) and 0.1M NaCl (*Sigma-Aldrich*) (1st layer) and 0.05M DEX (2nd layer) in Milli-Q water of 18 MΩ.cm resistivity. The films were synthesized by applying a constant potential of 0.8V (*vs* Ag/AgCl) and automatically stopped when the desired charges were reached (in general 50mC/cm² for PPy/Cl and 100mC/cm² for PPy/DEX). The bilayer films were then rinsed several times with Milli-Q water.

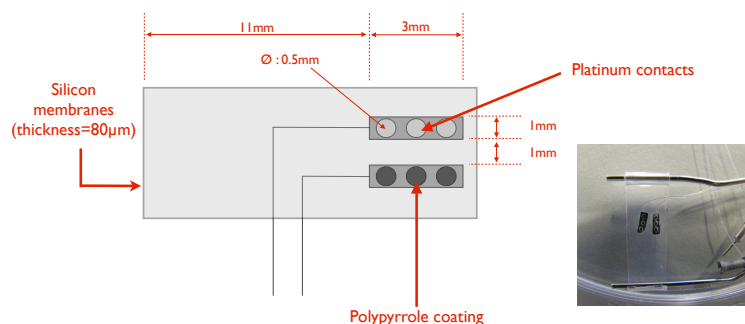


Figure 4.3: Cuff electrode (home-made fabrication: MOEX lab, UCL) covered with two-layer polypyrrole films.

4.2.3 Liberation of dexamethasone from polypyrrole films

① Electrical stimulation of PPy/DEX nanopillar brushes was performed by cyclic voltammetry (CV) using an *Egg Princeton Applied Research 273A* potentiostat. For drug release studies, the electrolyte was a 20mM PBS with 150mM NaCl (pH 7, room temperature). The voltage was switched from -0.8 to 0.9V at a scan rate of 100mV/s. The amount of released DEX was quantified by UV absorbance at 242nm (a characteristic band of DEX) using a *Cary 500 scan* UV-vis-NIR. The readings were taken after every five cycles until 50 cycles, then after every 10 cycles until 100 cycles and finally after 150 cycles. To ensure that the release is mainly caused by the electrical stimulus, a control was set up in which each sample was simply immersed in PBS solution (without any cyclic potential stimulus) and UV measurements were done after 10, 20 and 30min.

These results were compared with the first sets of CV cycles (considering for the x axis of passive release that 1 CV cycle corresponds to 1 min at 100mV/s).

② Electrical stimulation of two-layer PPy/Cl + PPy/DEX films was performed by potential pulses using a home-made *Neurotech* device (see figure 4.4) [22]. The device is connected through three entries: working electrode (WE or E_1), counter-electrode (CE or E_2) and reference electrode (RE or E_{ref}). The apparatus is accessible through home-made softwares for the different functions of the apparatus (*Galva 1.6* and *Relargage 1.2*). The functioning is the following: a potential (or a current, on choice) is imposed between E_1 and E_2 , and the current passing through is measured. A second potential is measured between E_1 and E_{ref} . Using this disposition, we can ensure that most of the current is flowing between WE and CE, leaving the reference electrode unmodified (classical three-electrode system).

The first software (*Galva 1.6*) was designed for the distribution of biphasic potential pulses. The

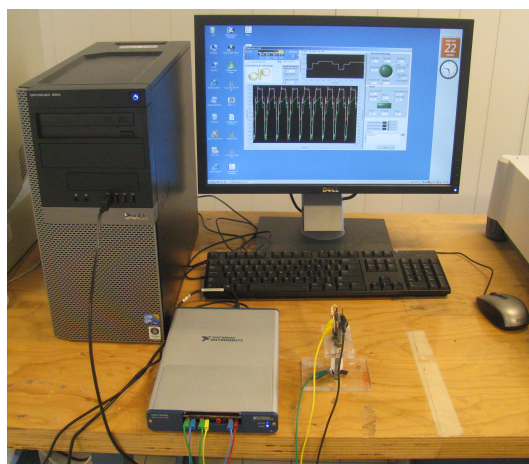


Figure 4.4: *Neurotech* device for potential pulses application.

parameters that can be changed are related to the pulse characteristics (times and potentials for the reduction, oxidation, interpulse steps) and the length of the stimulation (number of pulses, frequency).

The second software (*Relargage 1.2*) was designed for the distribution of biphasic potential pulses with an automatic following of the potential and the recovery of charge. This particular function first allows the automatic following of the potential based on the user choice. This set-up is a feed-back loop based on the comparison of the previous potential measurements versus the reference electrode, in order to avoid overoxidation of the polymer. The recovery of charge is based on the measurement of the current passing through the electrode during the reduction phase (based on user entry) and the automatic integration of the charge during the following oxidation phase, the oxidation being automatically switched off when the same charge is obtained. The parameters for the stimulation are summarized in table 4.1.

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Table 4.1: Parameters for biphasic potential pulses stimulation.

E_{red}	potential during the reduction pulse
E_{ox}	potential during the oxidation pulse
t_{red}	duration of the reduction pulse
t_{ox}	duration of the oxidation pulse
t_{inter}	duration of the rest inter-pulse phase between reduction and oxidation
f	frequency of the pulse cycle (pulses/second)

③ Electrical stimulation of two-layer PPy/Cl + PPy/DEX films-coated cuff electrodes was performed by potential pulses using the same *Neurotech* device (see figure 4.4). In this case, the reference was one of the platinum contacts (see figure 4.3). A single series of 1000 pulses was applied with the *Relargage 1.2* software ($E_{red} = -0.45V$, $E_{ox} = 0.3V$, $t_{red} = t_{inter} = 200\mu s$, $t_{ox} = 500\mu s$, $f=50Hz$).

4.2.4 SEM microscopy observations

The dimensions (height and diameter) of the Pt nanopillars were determined by SEM using a high resolution FEG Digital Scanning Microscope 983 Gemini from Leo, operating at 1kV. The morphologies of nanostructured electrodes coated with a PPy/DEX film were also observed by SEM using the same equipment.

4.2.5 Analysis of redox properties by EQCM

EQCM analysis of polypyrrole and copolymer films were carried out by cyclic voltammetry in a one-compartment teflon cell with a Pt wire as counter electrode and an *Ag/AgCl/sat.KCl* reference electrode using a *ChInstruments Model 400B* potentiostat with Time-Resolved Electrochemical Quartz Crystal Microbalance. Quartz crystals have a fundamental frequency of ~ 7.995 MHz and are coated with thin gold films on both sides. The gold disk deposited on the top side of the crystal is in contact with the electrolyte solution and used as the working electrode ($A=0.2cm^2$). CV experiments were done between +0.5V and -0.5V at a scan rate of 100mV/s, after the application of a constant potential of 0.5V for 10s, either in 0.1M NaCl or in ultra pure water. The density of the quartz (ρ_q) is $2.684g.cm^{-3}$ and the shear modulus of the quartz (μ_q) is $2.947 \cdot 10^{11} g.cm.s^2$.

4.2.6 Electrochemical Impedance Spectroscopy analysis

Impedance characterizations of gold flat surfaces and polypyrrole were performed using a *ChInstruments Chi660B* potentiostat in the same home-made three-electrode cell arrangement

as for synthesis. An *Ag/AgCl/3M NaCl* electrode (*Bio-Logic*) and a spring-like Pt wire (23cm, *Bio-Logic*) were used as RE and CE, respectively. The amplitude of the ac voltage was 5mV. Measures were done at 0.6V after applying the potential for 10s. The data measured in 0.1M NaCl were analyzed with the software *ZView* (Version 3.3a, *Scribner Associates Inc.*).

4.3 Results and discussion

4.3.1 Dexamethasone electrically controlled release from polypyrrole-coated nanostructured electrodes by cyclic voltammetry

As a proof of concept of the delivery of dexamethasone from nanostructured structures in a controlled and local way, the study of the synthesis and liberation and their influencing factors was done in a first time [1]. The hereafter results and discussion can be seen somehow as an introduction to the study of liberation parameters and their effects, which will be explained in detail in a more *in vivo-oriented technique*, corresponding to small potential pulses.

4.3.1.1 Preparation and structural characterization of nanostructured polypyrrole-coated electrodes

The efficiency of the developed process, based on the hard templating method, for preparing nanostructured electrodes was assessed by analyzing the electrode surfaces at each step of the preparation using SEM. First, a nanopillar brush was synthesized by electrodeposition of platinum within the nanopores of a PC membrane. Figure 4.5 shows a typical picture of a brush of self-standing Pt nanopillars obtained after dissolution of the PC template¹. As expected, the diameters of the nanopillars are quite monodisperse and correspond to the pore diameter of the PC membrane used as template, that is $\sim 150\text{nm}$. The mean pillar height (h_p) is controlled by the electrical charge passing through the system during the synthesis. The potential was automatically turned off when the desired charge was reached.

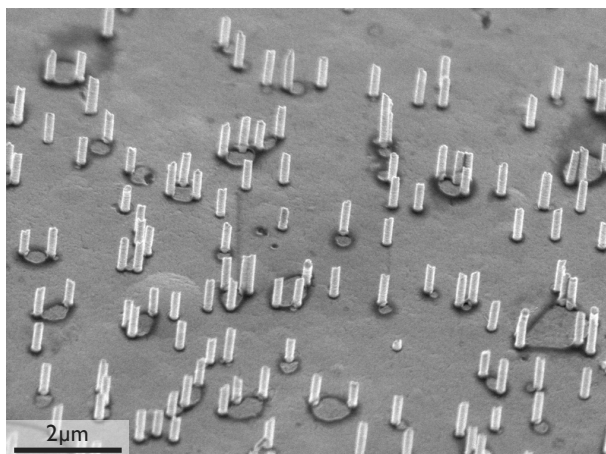


Figure 4.5: SEM picture of a typical brush of self-standing Pt nanopillars on a gold surface ($Q_{Pt} = 24 \text{ mC/cm}^2$, $\Phi_p \sim 150 \text{ nm}$, $d = 10^8 \text{ cm}^{-2}$).

In a second time, PPy/DEX films of various thicknesses were synthesized on the top of Pt

¹This picture and others of platinum nanowire arrays can be seen in details in the previous chapter of this work.

nanopillar brushes by a one step electropolymerization at 0.8V vs Ag/AgCl. As the growing PPy backbone chain is positively charged, the DEX ions present in the electropolymerization solution are incorporated during electrosynthesis in order to maintain the electroneutrality within the film. Figures 4.6 show typical images of the surface PPy/DEX films synthesized on a nanostructured electrode with different thicknesses. The thickness of the polymeric films can be roughly estimated from the electrical charge consumed during the electrosynthesis, by the relation: $30mC.cm^{-2}$ for a thickness of 50nm [8].

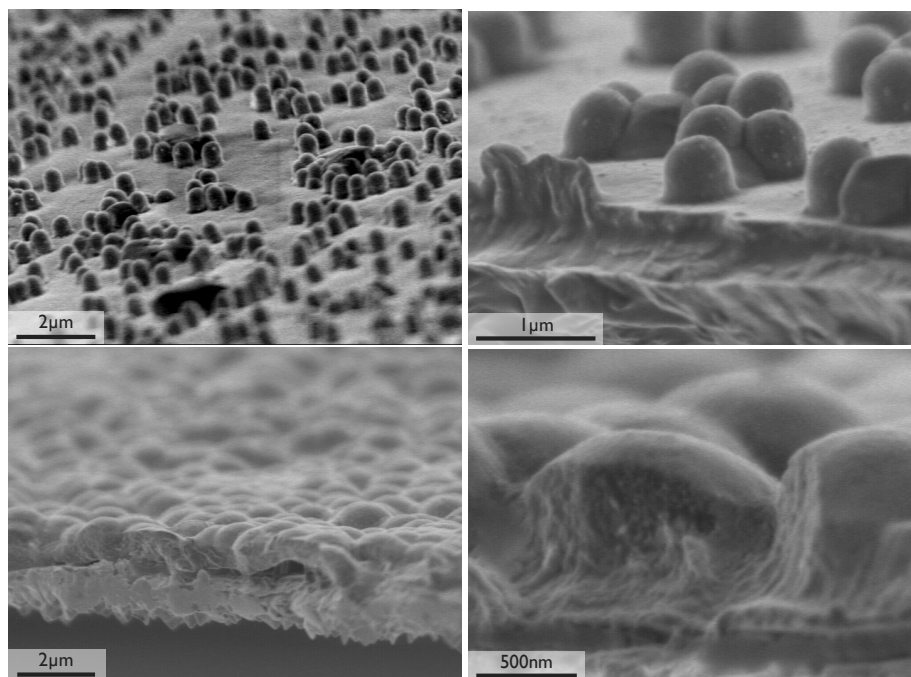


Figure 4.6: SEM pictures of typical brushes of self-standing Pt nanopillars ($\Phi_p \sim 150\text{ nm}$, $d = 10^8\text{ cm}^{-2}$) covered with PPy/DEX of different thicknesses (synthesis charge densities: $Q_{PPy} = 70mC/cm^2$ (up) and $Q_{PPy} = 350mC/cm^2$ (down)).

4.3.1.2 In vitro dexamethasone drug release study

The influence of the well-defined nanostructures established on the electrode surface on their release performances were then evaluated. Drug release from PPy/DEX films is based on the particular feature of PPy that can undergo redox cycling between conductive and insulating states. This redox switching is accompanied by swelling and shrinking of the polymer material due to the incorporation or release of hydrated ions A^- (figure 4.7).

In this work, A^- is the anionic DEX that was released from PPy/ DEX films by switching the potential between -0.9 and 0.8 V using cyclic voltammetry at a scan rate of 100 mV/s. Cyclic voltammetry is an electroanalytical technique that allows assessing the reduction and oxida-

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tion peaks of the polymer. Figure 4.8 presents the cyclic voltammograms (measured current against the applied voltage at the working electrode) recorded on a nanostructured PPy/DEX electrode after different number of scans. On figure 4.8.A, the switching properties of PPy can be clearly distinguished: two peaks appear at -0.2 and ~0.15V, corresponding to the reduction and oxidation of the nanostructured PPy/DEX film (figure 4.7), respectively.

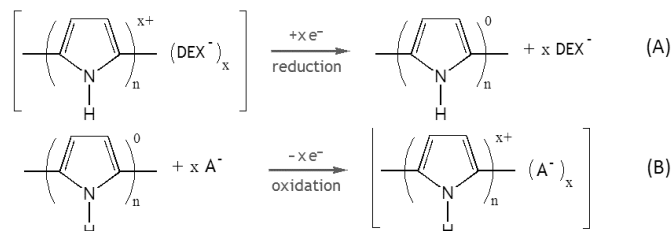


Figure 4.7: Reduction (a) and oxidation (b) reactions of a PPy/DEX film during electrical stimulation.

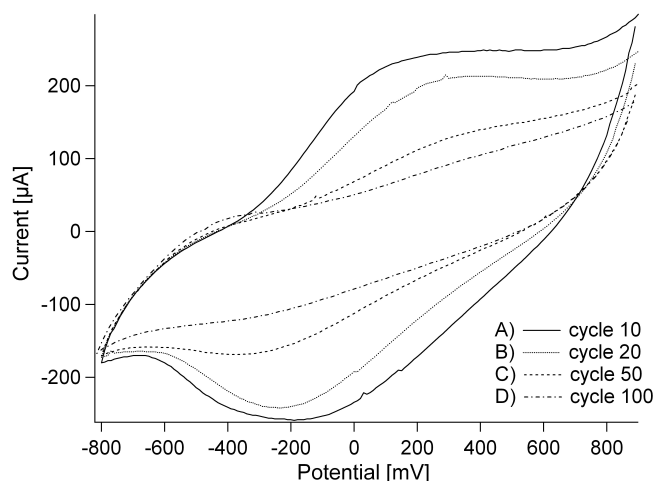


Figure 4.8: Cyclic voltammograms of a nanostructured PPy-DEX film ($Q_{PPy} = 39 \text{ mC/cm}^2$) recorded after 10, 20, 50 and 100 cycles of electrical stimulation. Scan rate = 100mV/s.

It should be pointed out that the redox peaks of PPy/DEX films on nanostructured electrodes appear at lower different potentials than on planar metallic electrodes, where, as reported by Wadhwa et al. [8], the reduction and oxidation peaks are located at -0.34 and ~0.49V, respectively. Our hypothesis is that these differences can be attributed to the influence of the Pt nanopillars on the PPy film electrosynthesis process and, consequently on its resulting morphology and electrical properties. These significant shifts of the redox potentials represent a serious advantage for the long-term performances of the electrodes, as lower applied potentials or currents could be used for the release of drug.

The release of DEX ions is however not an instantaneous process. To be detected, the anions have to diffuse from the inner part of the film to the surface and then, to the buffer solution.

The diffusion time of these bulky ions will depend on the PPy film density. Moreover, if the CV scan rate applied for the release is too high, DEX ions will remain in the PPy film during the potential switching and can therefore bind again to the positively charged polymer backbone upon oxidation, preventing the release of DEX in the solution. A too low CV scan rate could also bring some problems, as if the negative potential is applied for a too long time, the film becomes electrically insulating and, the recovery of the conductivity upon re-oxidation becomes less easier. It appears that cyclic voltammetry stimulation at a scan rate of 100mV/s is a good compromise between effective release properties of drug and preservation of the electrical characteristics of the film. At each CV cycle, a small amount of the total DEX ions contained in the PPy film is released.

The quantification of the DEX liberated was done using UV absorbance at 242nm. The cumulative DEX release profiles over 150CV for two different samples: a thin PPy/DEX film ($Q_{PPy} = 27.4mC/cm^2$) and a thick PPy/DEX film ($Q_{PPy} = 700mC/cm^2$) are shown on figure 4.9. In both cases, a first order relation ($R^2 \geq 0.97$) was found between the number of CV stimulation and the residual DEX amount, as presented in equation 4.9, where M_r [μg] is the residual quantity of DEX in the film after n cycles, M_r^0 [μg] is the initial quantity of DEX that can be liberated from the film² and k is a constant [$\mu g/cycle$].

$$\frac{dM_r}{dn} = -k.M_r \iff \ln M_r = \ln M_r^0 - k.n \quad (4.1)$$

At this point, M_r^0 was assumed to be equal to the released DEX amount after 150CV stimulation scans. This represents 39 and 106 $\mu g/cm^2$ for the thin and thick samples, respectively. Based on previously reported results [8], this is a sufficient amount of release DEX to significantly reduce the inflammatory tissue reaction in the vicinity of the electrode. As a control, PPy/DEX coated electrodes were simply immersed in PBS and the amount of DEX liberated was measured in function of time (so-called passive release). As expected and shown on figure 4.9, the amounts of DEX released by simple diffusion (passive release) were very low and negligible compared to the amounts released by electrical stimulation (assuming that 1CV at 100 mV/s corresponds to ~1 min). This indicates that our nanostructured PPy based drug release electrodes are true electrically driven release systems.

In order to study the influence of the PPy film properties on the release behavior of the nanostructured electrodes, samples with PPy/DEX films of various thicknesses were synthesized. As shown on figure 4.9, the released DEX amount increases with the film thickness, but the release profile remains identical. However, the quantity released does not linearly correlate with the film thickness. Here, the PPy/ DEX films were synthesized with the same current density, but a 25

²The initial quantity of dexamethasone inside the film is in reality different from M_r^0 because of the deeply-trapped ions, which can hardly be released out of the film (see state of the art).

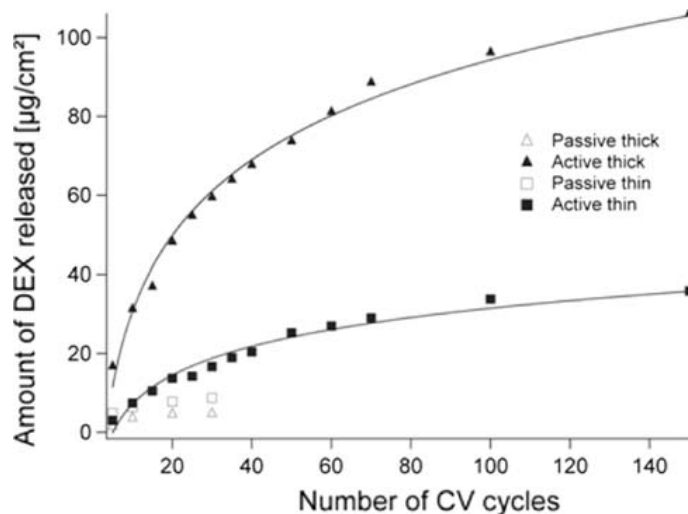
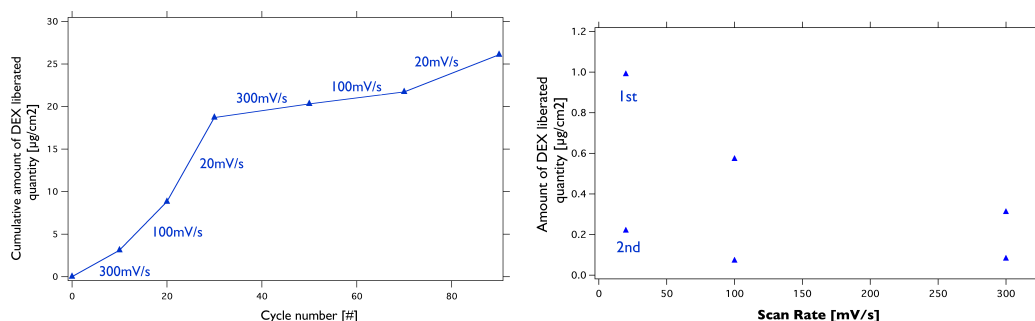


Figure 4.9: Release profiles of DEX from thin (filled square, $Q_{PPy} = 27.4 \text{ mC/cm}^2$) and thick (filled triangle, $Q_{PPy} = 700 \text{ mC/cm}^2$) nanostructured PPy films, determined by UV spectroscopy at 242nm. The drug release follows a first order kinetics. The x axis represents the number of CV cycles at 100mV/s for the active release and the time for the passive release (assuming that 1CV takes ~1min at 100mV/s).

times increase of the electrical charge (from 27 mC/cm^2 for the thin sample to 700 mC/cm^2 for the thick sample) corresponds to only a three times increase of the liberated DEX amount after 150 CV cycles. This can be due to the fact that PPy releases drug most efficiently from its surface, as opposed to from within the polymer bulk at the chosen rather high CV scan rate (100mV/s). We, indeed, observed that the scan rate influences the release profile, as can be seen on figure 4.10. It can be observed that when decreasing the scan rate on the same sample, the released DEX amount per cycle increased (figure 4.10.a and 4.10.b), and the film was more rapidly drained of DEX. This thus represents another way of controlling the amount of DEX released by the mean of varying the scan rate. This is a logical evolution, as the time to complete one cycle is higher when SR is small, involving a longer application of the reduction potentials, leading to a bigger amount of DEX liberated per cycle. We also observe a decrease of the quantity liberated per cycle when the number of cycle is higher, as the result of the previous liberation from the film (which follows a first order trend, as previously described). The evolution of electroactivity is the same as before, with a huge decrease over cycles, probably due to overoxidation of the film.

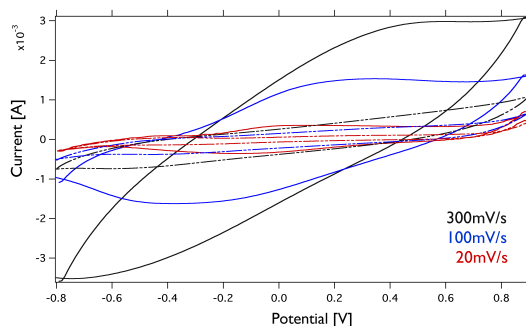
4.3.1.3 Structural and electrochemical characterization of PPy/DEX nanostructured electrodes after drug release

The morphology of PPy/DEX films on nanostructured electrodes were observed by SEM after 150 CV stimulating cycles of release. In literature, it was shown that conventional planar PPy films present cracks and delamination after a few number of cycles [8]. In our studies, the surface of nanostructured PPy/DEX films appeared similar before and after DEX release. Nevertheless,



(a) Evolution of DEX liberation from a plane PPy/DEX on gold ($Q_{\text{PPy}} = 500 \text{ mC}/\text{cm}^2$) in function of the number of CV cycles at different scan rates.

(b) Comparison of DEX liberation per 10CV cycles from a plane PPy/DEX on gold ($Q_{\text{PPy}} = 500 \text{ mC}/\text{cm}^2$) in function of the scan rate (first and second liberation).



(c) Evolution of PPy/DEX electroactivity of a plane PPy/DEX film on gold ($Q_{\text{PPy}} = 500 \text{ mC}/\text{cm}^2$) in function of the number of CV cycles at different scan rates (— CV10; --- CV50; — CV20; - - - CV70; — CV30; - - - CV90).

Figure 4.10: Influence of scan rate of cyclic voltammetry on liberation profiles of dexamethasone from plane PPy/DEX films.

we did not observe the initiation of cracks on planar gold surfaces covered with PPy films. However, the delamination of the polypyrrole film was sometimes observed while on planar gold electrodes, and did not appear on nanostructured surfaces. This possible improvement of the mechanical stability of the PPy film and of the adherence between the polymer and the metallic electrode substrate, that should help to lengthen the lifetime of the implanted electrode, can be attributed to the better anchoring of PPy film by the Pt nanopillars. Finally, we should mention that though the initial electroactivity of PPy/DEX film is increased by the nanostructuration of the metallic electrode substrate, we still observe, as on planar metallic electrodes, a decrease of the electroactivity upon DEX release (figure 4.8.B-D). This progressive

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loss of electroactivity probably occurs partly through the loss of doping molecules, but it is also probable that the stimulation over this range of potential induce a progressive overoxidation of the film, leading to a loss of its electroactivity. An optimization of the synthesis parameters in order to get stable electrochemical properties of PPy films over time was thus necessary, as this is a required condition for prolonged release applications.

4.3.2 Optimization of synthesis structure and parameters

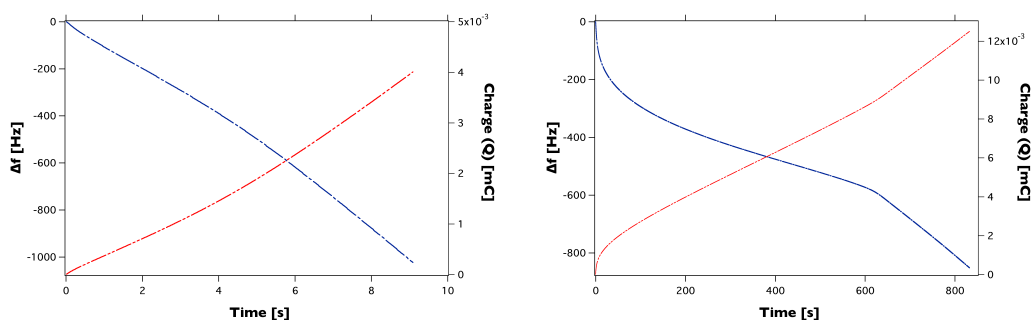
During this thesis, we showed the importance of several parameters on the synthesis efficiency of PPy/DEX films. Actually, the oxidation potential applied in this work was used in order to avoid overoxidation of the polymer during the synthesis. Wadhwa et al. [8] used higher potentials for their experiments, which is not suitable in our case. While using this kind of low potentials (0.8V *vs* Ag/AgCl here), the control on synthesis parameters is not perfect. In fact, the time needed to reach the desired charge density cannot be controlled (and thus the mean current density for the synthesis). This parameter greatly affects the properties of the films obtained, mainly in their density ; a low current density leading to more dense films, and the other way round [3, 23–25]. This significantly affects the liberation characteristics, with a variation of the diffusion properties of the counterions inside of the polymer. Still, some solutions are considered in literature to overcome this limitation. Thompson et al. used a two-layer approach for the synthesis of polypyrrole films containing neurotrophin molecules of large size [26]. The deposition of a thin continuous polypyrrole layer as the bottom of the electrode on which the layer containing the molecule of interest is synthesized presents several advantages. According to them, this approach may improve polymer growth by shielding the gold surface from the biomolecule, or may have an effect due to the increased surface roughness, and thus higher surface area of the electrode area.

In our work we used the same two-layer approach to overcome some reproducibility problems linked to the surface condition. By synthesizing a thin layer of polypyrrole with a small counterion (PPy/Cl) as a first layer, it lead to reduced and more similar synthesis times for the afterward synthesis of PPy/DEX films. The details for the synthesis parameters can be found in the materials and methods section.

4.3.2.1 Analysis of bilayers deposition by EQCM

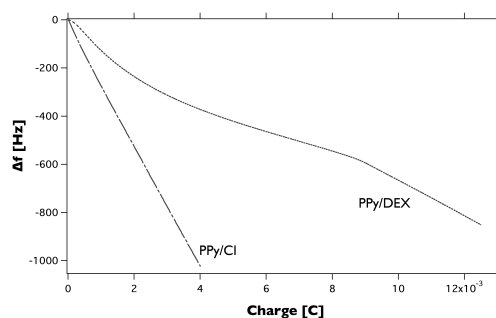
Polypyrrole films were synthesized in the form of double layers (or bilayers) on gold coated EQCM quartz crystals, as explained in the materials and methods section. Figures 4.11 show plots of the resonant frequency changes of the quartz resonators during the electrochemical synthesis of PPy/Cl and Py/DEX polymer films as first and second layers respectively. The negative resonant shifts (Δf) with time (and charge) indicates a deposition of matter on the gold coating of the quartz crystal. Moreover, the relation between Δf and the charge passed

(ΔQ) is quite linear, indicating an homogenous and linear film growth under the experimental conditions used in this work. For PPy/DEX, there is a small deviation from linearity, maybe due to a variation in the synthesis potential during the growth of the film. The films obtained are dark coloured and homogenous.



(a) Growth of PPy/Cl film monitored using EQCM (0.8V *vs* Ag/AgCl ; 0.1M Py + 0.1M NaCl ; 25mC/cm²).

(b) Growth of PPy/DEX film as second layer of a two-layers film monitored using EQCM (0.8V *vs* Ag/AgCl ; 0.1M Py + 0.05M DEX ; 50mC/cm²).



(c) Δf vs Q plots for the growth of PPy/Cl and PPy/DEX films of a two-layers structure monitored using EQCM.

Figure 4.11: Frequency-time (---), charge-time (---) and frequency-charge(---) plot for the growth of polypyrrole two-layers films.

EQCM measurements of polypyrrole synthesis (at each step of the formation of bilayers) were also used along with equation 4.2 (derivated from Sauerbrey equation, see 4.3) to calculate the corresponding actual value of the doping level (n) (Q is the charge for polypyrrole synthesis, Δm the change in mass per unit area during synthesis, F the Faraday constant, M_{Py} is the molar mass of pyrrole, M_A the molar mass of the incorporated counter-ion and M_H the molar mass of

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hydrogen). No neutral species entry during synthesis is considered in this equation.

$$n = 2 + y = \frac{Q}{\Delta m F} (M_{Py} + yM_A - 2M_H) \quad (4.2)$$

Table 4.2 presents the average frequency and mass variations during synthesis of each layer of the synthesized electrodes. The charge are two times lower than usual samples, as the conditions for the use of Sauerbrey equation has to remain valid. We observe that the calculated value for the doping rate of PPy/Cl is 0.47, which is theoretically impossible ($n \leq 0.33$). This could be due to a small error of measurements. We can thus make the hypothesis that the doping rate of the inner layer is near the theoretical value. However, in the case of the second polypyrrole layer, the doping rate appears to be negative, which is also impossible. The explanation can be found in the fact that the utilization of equation 4.2 postulates the use of every electric charge for the synthesis of polypyrrole (*ie* the current efficiency for the electropolymerization of the monomer unit is maximum). The poor electrical conductivity of the monomer solution with dexamethasone can also contribute to this low efficiency of polypyrrole synthesis. The effective layer thickness is thus probably lower than for a simple flat polypyrrole film.

Table 4.2: Frequency and mass variations, time and doping rate for the synthesis of polypyrrole two-layers films measured by EQCM.

Sample	Charge [mC/cm ²]	Time [s]	Δf [Hz/mC]	Δm [ng/mC]	Thickness vs charge	Doping rate
PPy/Cl	25	9.6 ± 1.2	- 256 ± 4	343 ± 5	40mC/cm ² for 100nm	0.47
PPy/DEX (2 nd layer)	50	1031 ± 246	- 80.6 ± 20.5	108 ± 27.4	60mC/cm ² for 100nm	- 0.0875

4.3.2.2 Analysis of bilayers redox properties by EQCM

EQCM measurements are widely used to follow the charging and discharging reactions occurring inside a conducting polymer [27–30]. The link between the change in oscillator frequency of a crystal and the change in mass on the crystal is given by the Sauerbrey equation, where Δf is the observed frequency change, Δm the change in mass per unit area, and C_f the sensitivity factor for the crystal, f_0 the resonant frequency of crystal's fundamental mode, A the area of the gold coated on the crystal, ρ_q the crystal's density and μ the shear modulus of quartz [31, 32] (see materials and methods for values).

$$\Delta f = C_f \cdot \Delta m \quad \text{where} \quad C_f = \frac{-2f_0^2}{A\sqrt{\mu\rho_q}} \quad (4.3)$$

The synthesized polypyrrole films must fulfill certain conditions in order to properly apply Sauerbrey's equation. The film should be rigid (perfectly elastic), thin and uniform. Film thickness should be small compared to the thickness of the crystal and the mass loading should

result in a small relative frequency change compared to the resonant frequency ($<2\%$) [31, 33–39]. For polypyrrole, Sauerbrey's equation was shown to be valid for thicknesses under $1\mu\text{m}$ [40] or even 500nm [31, 35]. In our case, $C_f = 7.4 \cdot 10^8 \text{ Hz/g}$ (or the net charge change of 1Hz corresponds to 1.35ng of material adsorbed on a crystal surface area of 0.196cm^2).

An examination of the flux of ions during potential cyclings of PPy films was carried out and surveyed by EQCM on two-layer systems in 0.1M NaCl . Figure 4.12 shows the results of these measurements, at different cycle numbers. The conditions for the application of Sauerbrey are met, regarding the estimated thickness of each layer. For PPy/Cl, we used the estimation from literature [41, 42] (and verified in our case by SEM observations [43]) with a value of $0.4\text{C cm}^{-2}\mu\text{m}^{-1}$ for PPy/ ClO_4 and PPy/ TSO^- coatings. This corresponds to a thickness of $\sim 62.5\text{nm}$ for a charge density of 25mC/cm^2 . For PPy/DEX films, we used the estimation measured by Wadhwa et al. [8] with a value of 50nm for a charge density of 30mC/cm^2 . In our case, the estimated thickness is thus $\sim 85\text{nm}$ for a charge density of 50mC/cm^2 . The addition of both thicknesses from the two layers is still inferior to the limit of use for the Sauerbrey equation.

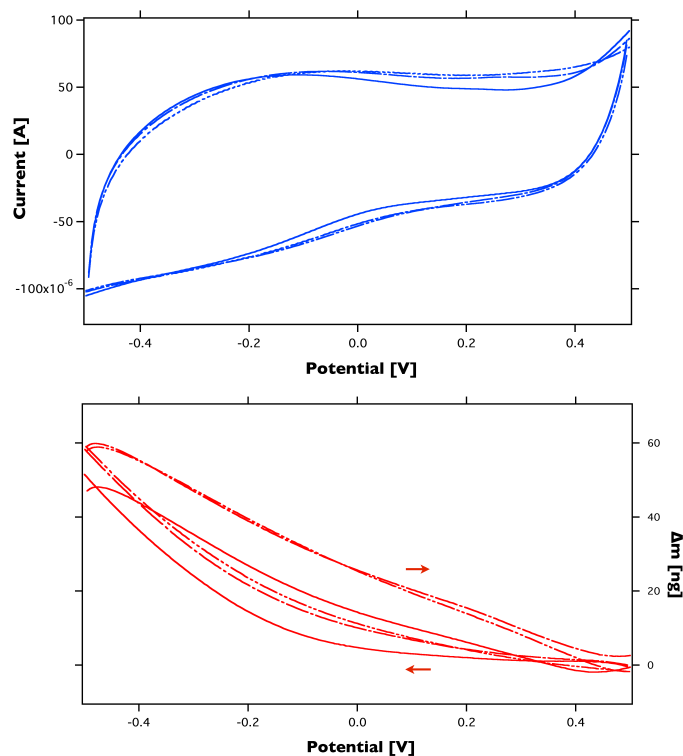


Figure 4.12: Current and mass variations accompanying oxidation and reduction of two-layer films immersed in 0.1M NaCl (PPy/Cl: 25mC/cm^2 ; PPy/DEX: 50mC/cm^2). The potential range was from 0.5V to -0.5V , and the scan rate 100mVs^{-1} (scan number 2 (—), 12 (---) and 52 (- - -)).

The first observation is the conservation of electroactivity over an important number of cycles. Compared to previous observations, it is attributed to the restriction of the potential window

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in order to avoid overoxidation. Regarding mass variations in function of the potential, the behaviour is a little bit counter-intuitive. We observe a clear increase in mass during the reduction sweeping of potential, appearing at 0V on cycle 2 and at even higher potentials on cycles 12 and 52. This corresponds to a charge compensation process dominated by the insertion of cations. On the re-oxidation direction, we observe the opposite mass variation, corresponding to the extraction of cations from the polypyrrole two-layers system. At the end of the cycle, the mass seems to be the same as at the beginning. On certain samples, a clear increase of mass after each cycle appears. This is attributed to the difference in the oxidation and reduction kinetics, the oxidation process being slower than the reduction one [31, 37]. This variation of mass highlights the mixed comportment of charge compensation on polypyrrole films containing dexamethasone dopants (medium/relatively low ion size), with the effective UV observation of dexamethasone controlled and sustained delivery from polypyrrole on one side, and the mostly cationic behaviour for charge compensation process. This has to be taken into account for the overall analysis of DEX liberation over time and the effect of the parameters on liberation from polypyrrole.

Assuming that cations are introduced during reduction, the theoretical mass associated with their movement can be calculated from the electrical charge needed for this reaction. Based on Faraday's law, the charge required for the neutralization of the doped polypyrrole units is given by: $Q_I = n_M F$; where n_M is the molar quantity of the cations involved in the charge movements. It can be written as: $Q_{C^+} = \frac{m_{C^+} F}{M_{C^+}}$; where m_{C^+} is the mass of the cation C^+ exchanged and M_{C^+} its molar mass. This is a theoretical value, assuming that all charges are used in the redox switching process. EQCM measurements provide the mass substituted from - or given to - the film. Combining both equations leads to relation 4.4, where $M_{app}^{C^+}$ is the apparent molar mass of species involved in the charge transport, ΔQ_{C^+} is the charge consumed during reaction and z the charge on the transferred ion.

$$\Delta m_{C^+} = \frac{M_{app}^{C^+} \Delta Q_{C^+}}{zF} \quad (4.4)$$

Figure 4.13 shows the masse variations *vs* the charge during redox CV in 0.1M NaCl for two-layers systems. It can be seen that in both directions, the mass variations go in the sense of a decrease with charge increment. This confirms the cationic nature of the charge compensation process³.

As described by Li et al. [44], who investigated polypyrrole films doped with a variety of dopants (namely chloride, nitrate, para-toluene sulfonate and dodecyl sulfonate) by means of cyclic voltammetry in NaCl, NapTS, and $AlCl_3$ aqueous solutions, the CV current measurement generally display four ionic exchange processes. Those processes can be represented by a

³As a simplification, the overall behaviour is analyzed, without the decomposition of each layer's effect on mass variation over potential. This will be done in another chapter. The general development is still valid taking this approximation into account.

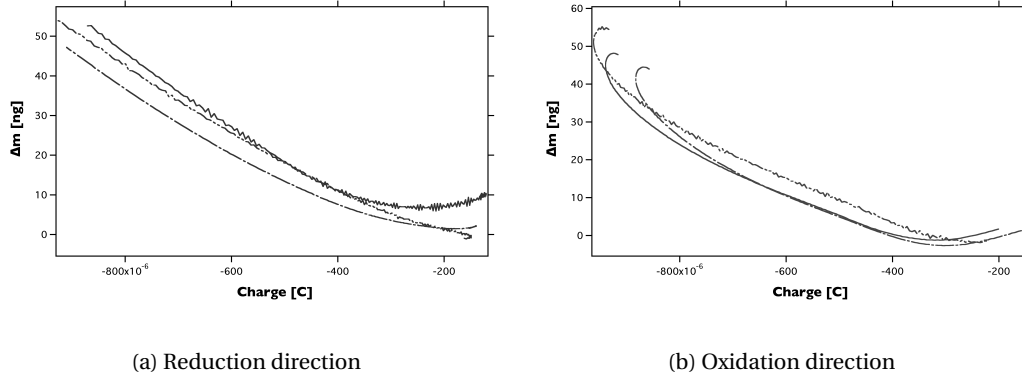
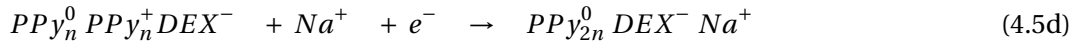
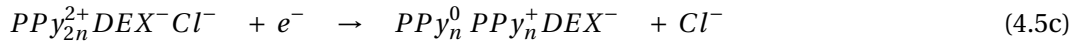
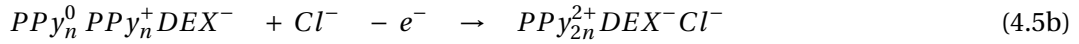
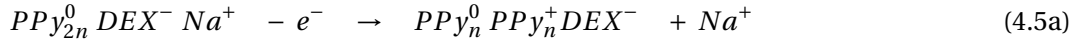


Figure 4.13: Mass changes as a function of charge during CV cycling (scan number 2 (—), 12 (---) and 52 (---)) of bilayers systems in 0.1M NaCl (PPy/Cl: $25\text{mC}/\text{cm}^2$; PPy/DEX: $50\text{mC}/\text{cm}^2$).

system of equations representing cation ejection, anion insertion, anion ejection, and cation insertion. If we implement these equations on the studied system, we obtain the following equations (see equations 4.5a, 4.5b, 4.5c and 4.5d).

In our case, only equations 4.5a and 4.5d can be observed for the redox behaviour (although a really small portion can be attributed to the reactions described at 4.5b and 4.5c at high values of charge).



On table 4.3 are calculated the respective values for $M_{app}^{C^+}$. It appears that the apparent molar mass for cationic exchange is smaller than the molar mass of the ions involved (Na^+ : $23\text{g}/\text{mol}$). We attribute this to the mixed comportment of PPy/DEX films during reduction, which thus cannot be described entirely and only by equations 4.5a and 4.5d. This complex phenomenon is thus concurrent to the liberation of DEX^- ions during reduction, as already described earlier in figure 4.7.

The EQCM analysis of bilayers redox properties presented here is a proof of the complex behaviour of dexamethasone liberation from polypyrrole, because on a large scale we observe a liberation of dexamethasone from the film (UV absorbance) and on a small scale a mixed comportment of charge compensation (EQCM). This has to be kept in mind in the further analysis of the effects of electrochemical parameters on dexamethasone liberation from polypyrrole.

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Table 4.3: Apparent molar mass ($M_{app}^{C^+}$ [g/mol]) determined from the respective slopes during cathodic and anodic sweeping of polypyrrole films immersed in aqueous 0.1M NaCl between 0.5V and -0.5V (100mV/s) (see figure 4.13)

Ions	Cycle	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (cathodic direction)	$M_{app}^{C^+}$ [g/mol]	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (anodic direction)	$M_{app}^{C^+}$ [g/mol]
$\text{Na}^+ ; \text{Cl}^-$ [0.1M]	2	94	9.1	68	6.6
	12	83	8	67	6.5
	52	86	8.3	66	6.4

Note: the same signs for oxidation and reduction half-cycles reflect the reversal in direction of both mass and charge flow. When mass and charge have the same (opposite) direction, the dominant species involved in charge transport is anions (cations).

4.3.2.3 Analysis of electrochemical properties of polypyrrole two-layers by EIS

Figures 4.14 and 4.15 show the EIS measurements for a two-layer polypyrrole film, at every step of the synthesis and after electrochemical stimulation by cyclic voltammetry. The corresponding current measurements during stimulation are also presented, to depict the electroactivity of the film. The impedance module is drastically diminished by the synthesis of a polypyrrole film on the gold electrode. This is a long-time observed effect in literature, attributed to the charge transport in polypyrrole compared to the flat metallic electrode [8]. As observed on this figure, the module of the two-layer structure is higher than the one of the simple PPy/Cl film. This is attributed to the presence of dexamethasone ions as dopants. Their relatively high size and poor conductivity engender bad charge transfer properties for these films, resulting in a high impedance module. This was already observed in literature [45–48]. We also notice the interesting fact that after a few CV cycles and the consecutive dexamethasone liberation, the impedance module is lowered. This can be attributed to the replacement of DEX^- ions by Cl^- ions after successive electrical redox stimulations, which lead to more conductive polypyrrole films as dopants (small size, quick diffusion). As observed on the corresponding current measurements during stimulation, the use of smaller potential intervals allow the electroactivity to remain at the same level, by avoiding overoxidation reactions.

4.3.3 Limitations of cyclic voltammetry for dexamethasone delivery from polypyrrole for *in vivo* applications

As it has been demonstrated in a simplified media, the liberation by cyclic voltammetry can be controlled while acting on several technique parameters, with the liberated quantity following a predictable trend *in vitro*. However, in the objective of using cyclic voltammetry in a more complex biological media or even *in vivo*, some limitations directly appear. First, we can mention the possibility for the appearance of parallel/side reactions in the potential window in which the redox reactions appear. This can induce injuries to the tissues in contact as well as damage

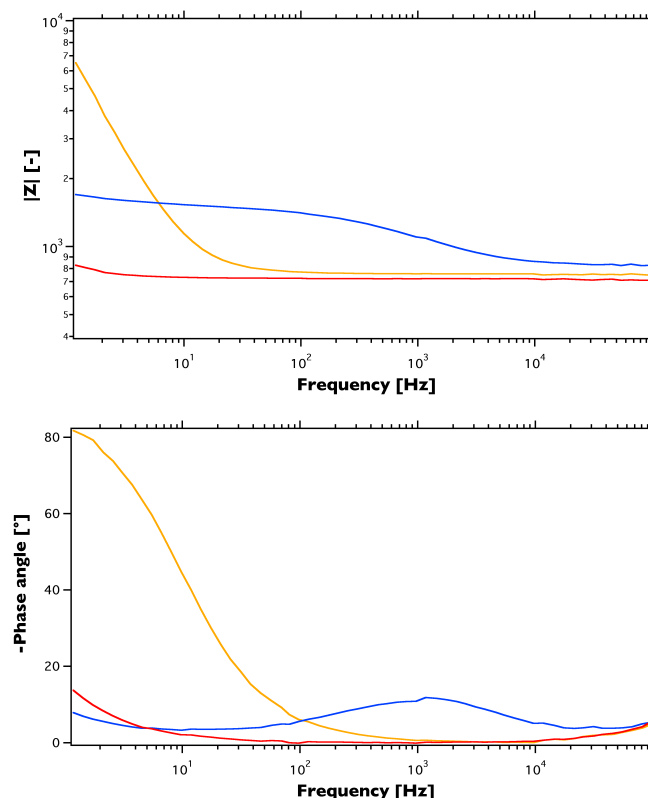
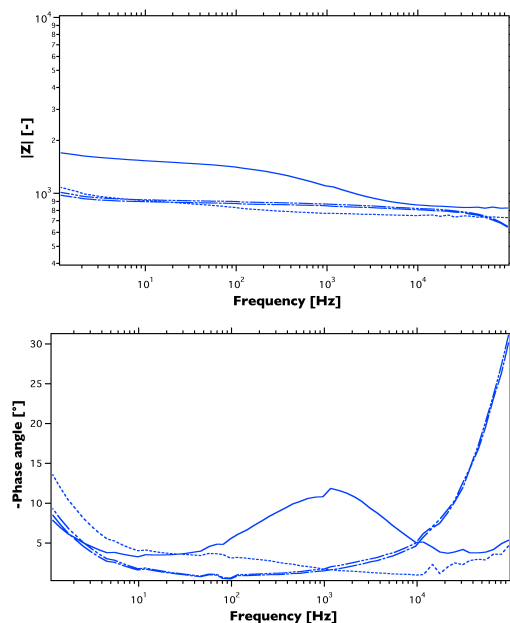


Figure 4.14: Bode plots of module and phase angle for bare gold (—), PPy/Cl (— ; 50mC/cm^2), PPy/Cl + PPy/DEX films before (— ; 100mC/cm^2) recorded in 0.1M NaCl using a perturbation amplitude of 5mV, measured at 0.6V vs Ag/AgCl.

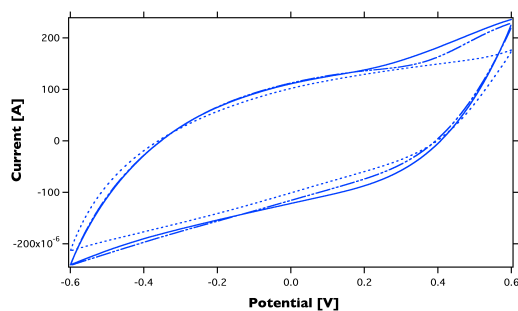
on the polymer film itself. Moreover, as the liberation has to be implemented on a long-term implanted material, it has to be compatible to the stimulation electronic (components and structure) and to the life-time of the carried-on battery. Withal, according to implant specialists [22], the implementation of a cyclic voltammetry technique is not the best choice for the power consumption, and in consequence not the right option to operate a local and controlled drug delivery from a stimulation implant.

In literature, some other techniques have been developed to perform such controlled drug delivery from polypyrrole films, as presented in the state of the art section (tables 2.25 and 2.26). In particular, we can distinguish the technique used by Richardson et al., Thompson et al. and Evans et al. [6, 7, 26, 49–53] with (charge-balanced) biphasic current pulses of short times ($\sim 100 - 250\mu\text{s}$) and relatively high frequency ($\sim 250\text{Hz}$). They demonstrated the controlled release of neurotrophin entrapped in polypyrrole films with a good control over the number of pulses (through the time of pulse application) and a clear quantitative difference between the stimulated and non-stimulated coated electrodes, in addition to the conservation of the original purpose of their cochlear implant, *ie* to deliver electrical current to spinal ganglion neurons for

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(a) Bode plots of module and phase angle for PPy/Cl + PPy/DEX films before (—) and after drug delivery by cyclic voltammetry ((-0.5 ; 0.5V), 100mV/s, 0.1M NaCl) (--- CV10 ; --- CV20 ; --- CV100) recorded in 0.1M NaCl using a perturbation amplitude of 5mV, measured at 0.6V *vs* Ag/AgCl.



(b) Evolution of the corresponding electroactivity of a plane PPy/Cl ($50mC/cm^2$) + PPy/DEX ($100mC/cm^2$) film on gold in 0.1M NaCl in function of the number of CV cycles (— CV10 ; --- CV20 ; --- CV100).

Figure 4.15: EIS and CV measurements for two-layer polypyrrole films at every step of film synthesis and stimulation.

auditory perception.

The evident problem in the use of this technique in our purpose of redox controlled liberation is the possibility to attain overoxidation potentials. Indeed, in their apparatus, there is no control of the applied electrochemical potentials on the polypyrrole films. This is critical for the integrity of the film in a long-term stimulation overview. In our case, the conservation of a good electroactivity over time is crucial, and the possibility to reach overoxidation cannot be tolerated.

Accounting for these requirements and limitations, the advised technique to fulfill all those conditions is the application of small potential pulses, in respect to a reference electrode. The technique as well as its parameters and the apparatus to perform it will be detailed in the next section.

4.3.4 Dexamethasone electrically controlled release from polypyrrole-coated electrodes by potential biphasic pulses

4.3.4.1 Description of the technique parameters

On figure 4.17 is described a so-called *pulse cycle* as used in this work to undergo drug delivery. A pulse cycle can be separated into three steps. It begins with the application of a reduction potential (E_{red} for a time t_{red}), during which the dopant ions are liberated from the polypyrrole film. An *inter-pulse* follows, during which no potential is applied (*ie* open-circuit, for a time t_{inter}). Finally, an oxidation potential (E_{ox} for a time t_{ox}) is administered. The reason for the biphasic character of pulse cycles is linked with both the necessity for polypyrrole to be re-oxidized in order to conserve its electroactivity, and the biological requirement to take back the electrical charges injected to the tissue. If not, the latter can induce damage to the surrounding tissues in addition to reduced tissue sensitivity (for recording or stimulation) [54]. The second parameters appearing on figure 4.17 are the durations of the pulses and inter-pulses. The latest is the frequency of the application of pulse cycles, which does not appear on this figure. The choice for frequency of application is primarily determined by biological limitations. All these characteristics are to be modulated in order to act on the liberated DEX quantities from the films.

The device for the application of potential pulses was described in the material and method section. The communication window for the software can be seen in figure 4.17. On this window, we can see on the upper right the parameters for the application of pulses (times, potentials and frequency). On this window are also pictured the current and potential measurements: the potential between working electrode and counter electrode (red), between working electrode and reference electrode (green) and the current between working electrode and counter electrode (white). While applying an electrochemical potential (*vs* reference), the potential parameters are introduced. These potentials are applied between the working electrode and the counter

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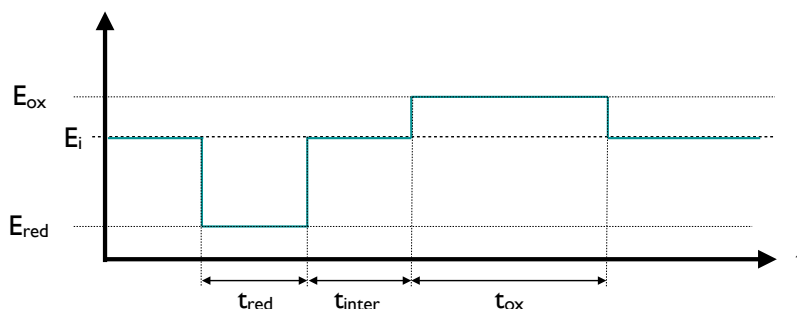


Figure 4.16: Description of a biphasic pulse cycle and its parameters as used for drug delivery.

electrode, as in a classic three-electrode system, in order to avoid current flowing through the reference electrode. The differential potential versus reference electrode is measured and at the same time the current, as aforementioned. The introduced parameters are then adjusted to obtain the aimed values of potential. The electrical charges consumed during the redox reactions are integrated from the currents displayed.

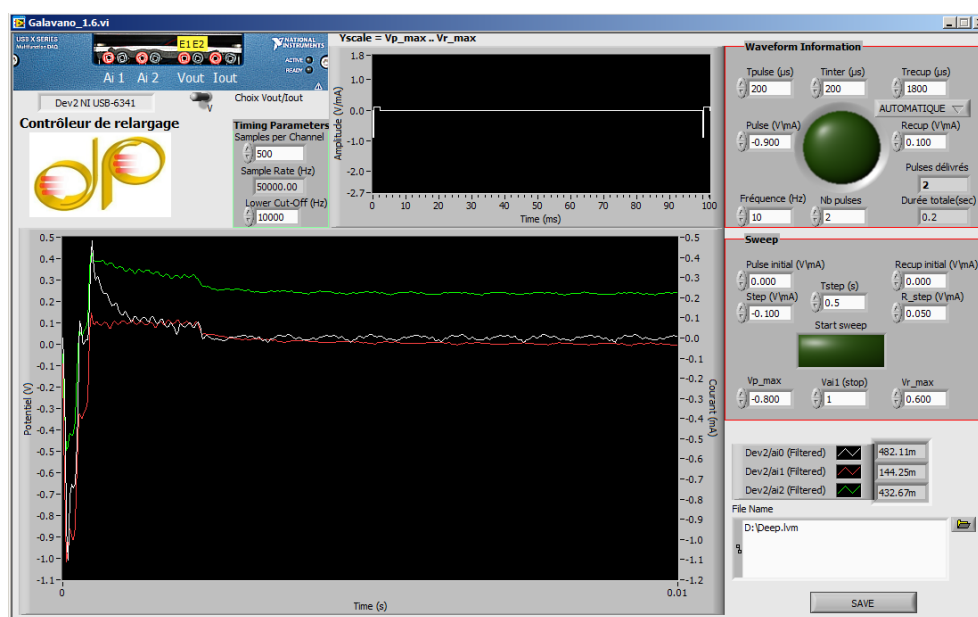


Figure 4.17: Software communication window for the application of potential pulses (version Galva 1.6).

Based on literature and experts collaborations, the following limitations have been fixed. First, the potential are chosen in the window such as overoxidation is avoided (and the other possible side reactions in a complex biological environment). Speaking of the inter-pulse time, its choice should take into account the time for anions to diffuse outside of the polymer membrane during and after reduction, and the biological limitation linked to the possibility to recover the electrical charge injected. The order of magnitude of the inter-pulse time is $100\mu s$ as a minimum and

$\sim 500\mu s$ at maximum. On time for the redox pulses depends the quantity of DEX released. This is one of the main parameters that was studied in the scope of this thesis. The frequency of pulse cycle is also tributary of biological restrictions. For the vagus nerve stimulation, the optimal frequency is around 50Hz, to be sure to avoid any kind of destruction of tissues.

In the next sections will be described the results for the analysis of biphasic potential pulses liberation. This represents a summary of the actual results obtained during this thesis. Some parameters were optimized simultaneously to the development of the stimulation device and the in vivo requirements and limitations. Not all data will be presented, only the most relevant in terms of liberation and in vivo applicability. Most particularly, the frequency and the duration of the pulses were drastically decreased in the course of this work.

In the following section will be presented the qualitative and quantitative study for the release of DEX from polypyrrole bilayer films, together with the analysis of the effect of the parameters on this drug delivery.

4.3.4.2 Liberation of DEX from bilayer films by potential pulses

In the liberation operated by cyclic voltammetry, we can first distinguish the passive liberation (*ie* without any electrical stimulation) and active stimulation (consecutive to an electrical stimulation by a certain number of pulse cycles). Those two liberation schemes will be developed.

4.3.4.2.A Passive liberation On figure 4.18 are shown the measurements for passive liberation of DEX in a PBS solution as a function of time, from polypyrrole bilayers (synthesis details in materials and methods). We can see that passive liberation occurs principally during the first hours of soaking, and afterwards reaches a plateau (after 50h). It is an important fact to know, because the passive liberation is a non-controlled phenomenon in quantity. It is thus important to clear the surface of it. The simplest solution is thus to soak the electrodes into a PBS solution for at least three days to ensure the passive release of all the dexamethasone simply adsorbed on the surface or physically entrapped into to polymer that does not necessitate a reduction reaction to diffuse out of the film.

4.3.4.2.B Active liberation

4.3.4.2.B.1 Active liberation as a function of time In the study of drug delivery from polypyrrole, the liberation of molecules from the films is considered instantaneous in literature. In our experiments, we observed a time-lag in the delivery between the stimulation and the effective release of dexamethasone. This time-lag takes the form represented in figure 4.19, correspond-

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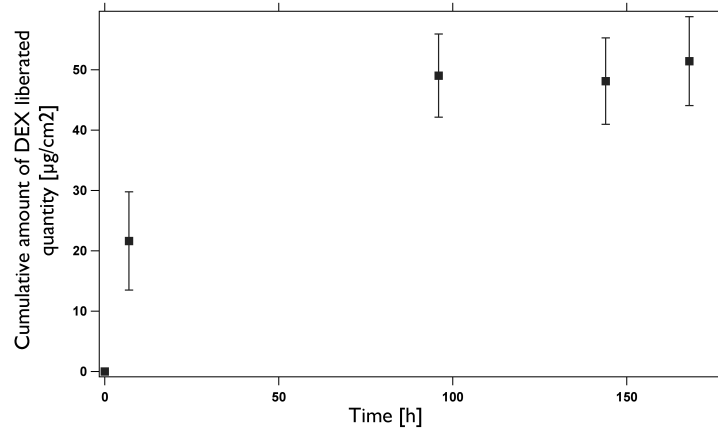


Figure 4.18: Passive liberation in function of time of DEX from polypyrrole bilayers (PPy/Cl ($50\text{mC}/\text{cm}^2$) + PPy/DEX ($100\text{mC}/\text{cm}^2$)) into a PBS solution ($\lambda=242\text{nm}$).

ing to the function in equation 4.6 ; where Q_0 is the maximal quantity of DEX accessible for the liberation from inside the film⁴, k is a constant and x is the time (or another variable, as the cycle number). This means that the liberation of the molecules continues even after the interruption of potential pulse stimulation.

$$f(x) = Q_0(1 - e^{-k \cdot x}) \quad (4.6)$$

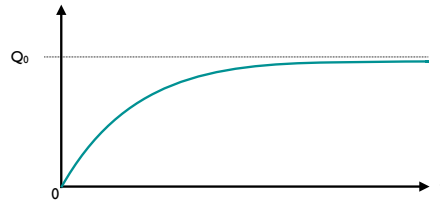


Figure 4.19: Graphical representation of the theoretical liberated quantity versus the time from a polypyrrole film after electrical stimulation.

Figures 4.20 show two examples of release of dexamethasone from plane polypyrrole two-layer systems, after long series of potential pulses. Different series of pulses are applied to the electrodes, with a passive (non-stimulated) period following each active stimulation. The release of dexamethasone continues for minutes to hours after stimulation, finally reaching a plateau

⁴The initial quantity of dexamethasone inside the film is in reality different from Q_0 because of the deeply-trapped ions, which can hardly be released out of the film (see state of the art).

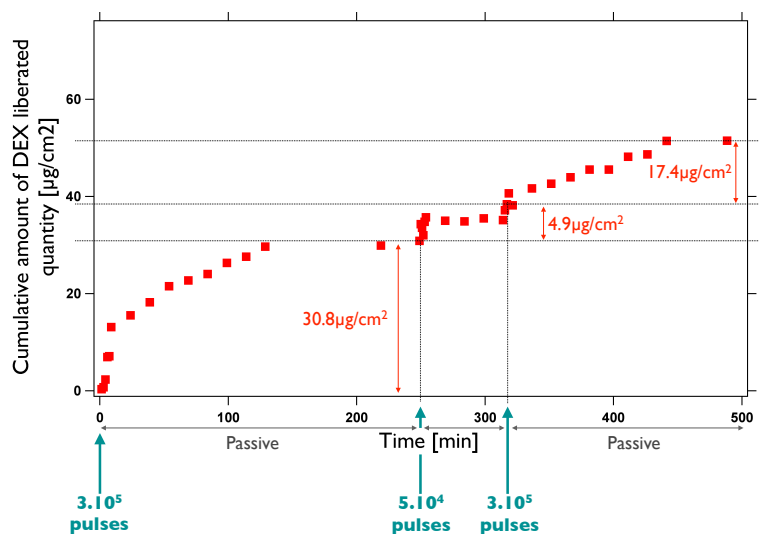
corresponding to the final quantity liberated in correspondence with the stimulation parameters applied.

On the first example, we can see that a stimulation of $3 \cdot 10^5$ pulses leads to a $30.8 \mu\text{g}/\text{cm}^2$ liberation. The second stimulation of $5 \cdot 10^4$ pulses leads to a $4.9 \mu\text{g}/\text{cm}^2$ liberation. Finally, the last $3 \cdot 10^5$ pulses lead to a $17.4 \mu\text{g}/\text{cm}^2$ release. We can conclude that increasing the number of pulses leads to a larger liberation, with a certain proportionality in the first time. The discrepancy after the third stimulation is probably due to the exhaustion of the film, corresponding to the same behaviour as for the liberation by cyclic voltammetry. The second example confirms this trend, with two stimulations of 10^5 pulses engendering the liberation of $19.4 \mu\text{g}/\text{cm}^2$ and $19.6 \mu\text{g}/\text{cm}^2$.

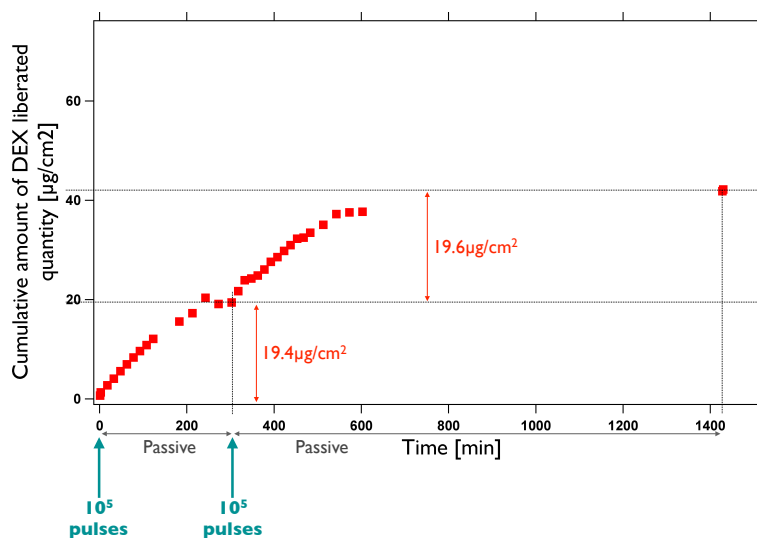
However, it has been observed that this proportionality between the number of pulses and the liberated quantity is not always achieved, even before the influence of film exhaustion. This can be attributed to the previously described lack of reproducibility between sample caused by differences in the densities of the films. This can also be seen on the calculated fittings based on equation 4.6, for the two previously presented examples (see figure 4.21). It can be seen that the characteristics for the liberation kinetics (k parameter) do not follow a general trend, even for the successive liberations from the same sample.

The conclusion that can be drawn from these data is that in the aim of in-vivo applications, the interruption of dexamethasone liberation is not instantaneous after the one of electrical stimulation. Using this observation, we studied the effect of small electrical stimulation separated by long periods of passive release.

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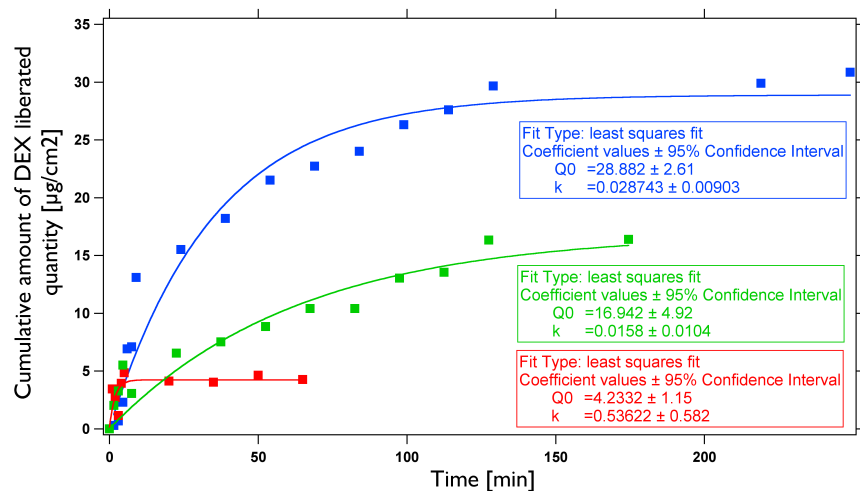


(a) Liberation quantity from a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) after potential pulses stimulation ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=900\text{Hz}$).

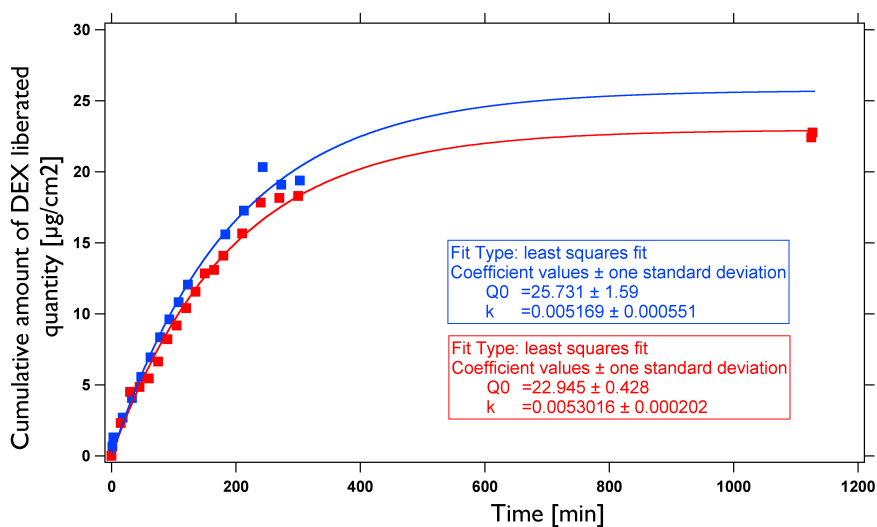


(b) Liberation quantity from a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) after potential pulses stimulation ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=900\text{Hz}$).

Figure 4.20: Liberation quantity from two-layer PPy films versus the time after long series of potential pulses.



(a) Fitted liberation quantity from two-layer films (PPy/Cl: 50 mC/cm^2 ; PPy/DEX: 100 mC/cm^2) after potential pulses stimulation ($E_{red} = -0.45 \text{ V}$, $E_{ox} = 0.3 \text{ V}$, $t_{red} = t_{inter} = 200 \mu\text{s}$, $t_{ox} = 500 \mu\text{s}$, $f=900 \text{ Hz}$) after 10^5 pulses.



(b) Fitted liberation quantity from a two-layer film (PPy/Cl: 50 mC/cm^2 ; PPy/DEX: 100 mC/cm^2) after potential pulses stimulation ($E_{red} = -0.45 \text{ V}$, $E_{ox} = 0.3 \text{ V}$, $t_{red} = t_{inter} = 200 \mu\text{s}$, $t_{ox} = 500 \mu\text{s}$, $f=900 \text{ Hz}$) after 10^5 pulses.

Figure 4.21: Fitted liberation quantity from a two-layer film, using equation 4.6 on samples 4.20.a (first (—), second (—) and third (—) pulse stimulation) and 4.20.b (first (—), second (—)).

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4.3.4.2.B.2 Active liberation: series stimulation *vs* continuous stimulation In literature, pulse stimulation (most of the time current pulses) is applied continuously for long periods of time, at a fixed frequency, the liberated quantity being measured as a function of time (up to several days) [6, 7, 26, 49–53]. In the aim of this project, we compared the effect of continuous stimulation compared to the application of a short series of pulses followed by long electrically inactive (passive) periods. Actually, it is better for the integrity of the tissues to use small series of stimulation pulses Figure 4.22 shows the comparison of liberation from polypyrrole films with large number of cycles (■) and short series (■). On this graph, we can see that the quantitative release of dexamethasone is better while using a short stimulation. On one side, a short serie of 10 pulses is applied and the cumulative amount of DEX released is measured over time up to a plateau, as described before. The quantity shown here is thus the quantity which can be obtained after a very short stimulation episode followed by passive release (■). On the other side, a constant stimulation (with the same parameters) is applied and the cumulative quantity of DEX measured after an important number of pulses (■). This proves the possibility to obtain a significant liberation of DEX with short series of pulses in comparison to continuous stimulation. Moreover, the advantage of it is to avoid uninterrupted nerve impulse and film redox solicitation. Additionally, the release of a second component appears when a stimulation of a large number of pulses is applied, as shown on figure 4.23. The shoulder emerging at $\sim 225\text{nm}$ is attributed to oligomeric (or polymeric) chains released from the film as a consequence of cracking and delamination. This critical damage to long-term electrode performances is a second argument to use small cycles of stimulation.

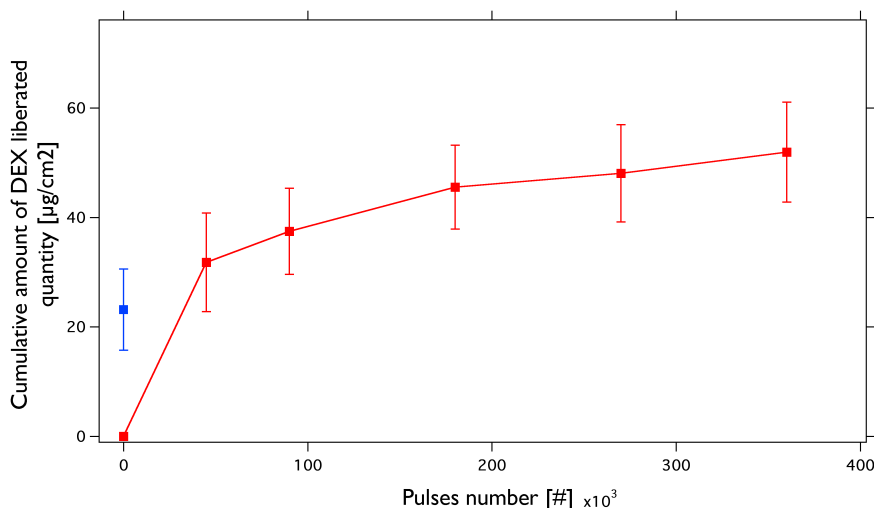
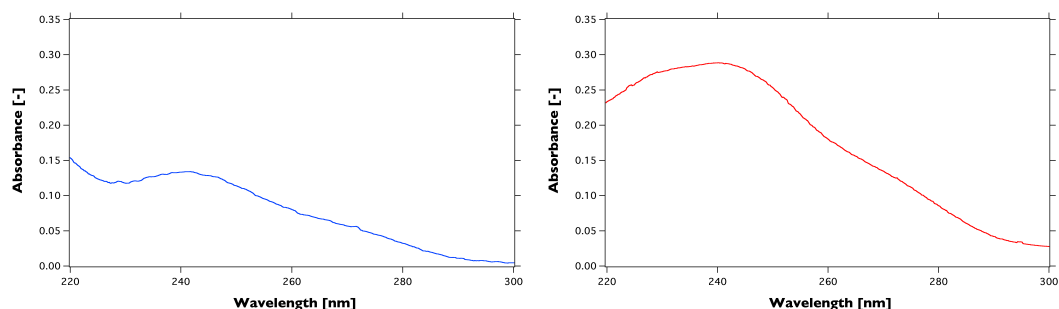


Figure 4.22: Liberation quantity from two-layer films (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) after potential pulses stimulation ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=50\text{Hz}$) as a function of pulse number for continuous (■) stimulation and a short 10 pulse series (■).



(a) UV absorbance measurements from two-layer films (PPy/Cl: $50mC/cm^2$; PPy/DEX: $100mC/cm^2$) after potential pulses stimulation ($E_{red} = -0.45V$, $E_{ox} = 0.3V$, $t_{red} = t_{inter} = 200\mu s$, $t_{ox} = 500\mu s$, $f=50Hz$) of a 10 pulses series.

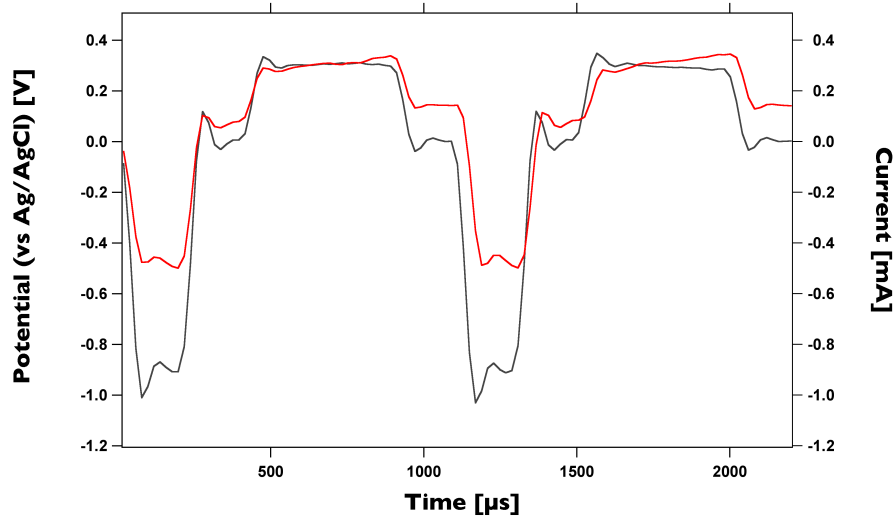
(b) UV absorbance measurements from two-layer films (PPy/Cl: $50mC/cm^2$; PPy/DEX: $100mC/cm^2$) after potential pulses stimulation ($E_{red} = -0.45V$, $E_{ox} = 0.3V$, $t_{red} = t_{inter} = 200\mu s$, $t_{ox} = 500\mu s$, $f=50Hz$) of a $3.6 \cdot 10^5$ pulses series.

Figure 4.23: Comparison of UV absorbance measurements from polypyrrole two-layers films with different stimulation parameters.

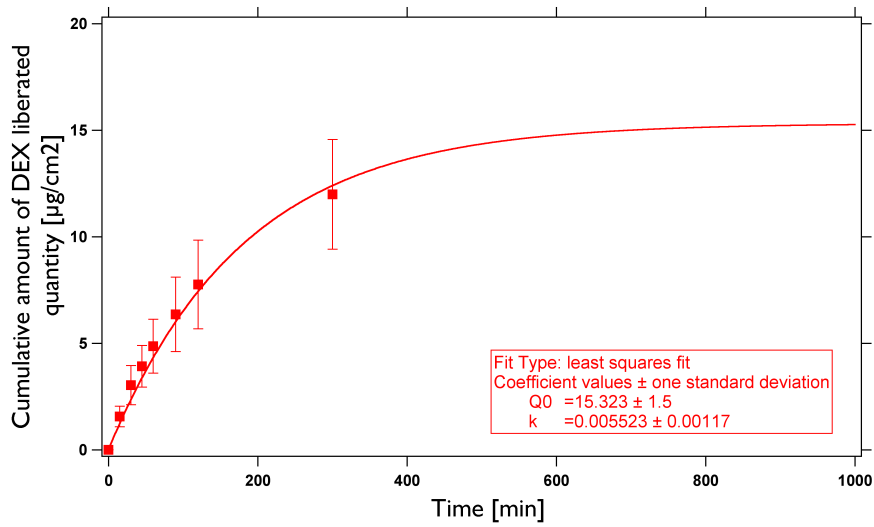
4.3.4.2.B.3 Active liberation: series liberation over time Knowing that the pulse series liberation is a best choice than continuous stimulation, we applied short series of 10 biphasic pulses and measured the passive liberation over time of dexamethasone from bilayer structures. The results can be seen in figure 4.24, with the potential and current measurements in 4.24 (a) and the corresponding drug release profile in 4.24 (b). The liberated quantity trend is the same as previously described, with the parameters of equation 4.6.

The stimulation was repeated over 4 days, with a series of 10 pulses each day. An example of liberation profile is presented in figure 4.25. On this profile, we can observe a *step release* after each series of pulses, with a decreasing quantity at each time. This is the ideal case observed on the samples. Over more samples, the description of a mean behaviour is less direct. We observe on each sample a *burst-release* in the passive liberation following the first series of 10 pulses. However, the mean release appears at this time. The quantity liberated by the next series is often not significant. We thus studied the application of larger numbers of pulses on the second day (after a 10 pulses liberation on day one), but the conclusion was the same. The important parameter for the quantitative control on the release of dexamethasone by biphasic current pulses is still to be determined.

Experiments were done in order to show the importance of the reduction potential. A qualitative variation: liberated quantity increased while reduction potential decreased. However, no quantitative relation was shown under a certain potential. Finally, the important parameter in the quantitative control of delivery seems to be the electrical charge, within the considerations of the influences by all the parameters studied in this work. A new liberation software is currently being developed to test the relation between charge and liberated quantity.



(a) Current (—) and potential (versus Ag/AgCl) (—) measurements during potential stimulation of a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) ($E_{\text{red}} = -0.45\text{V}$, $E_{\text{ox}} = 0.3\text{V}$, $t_{\text{red}} = t_{\text{inter}} = 200\mu\text{s}$, $t_{\text{ox}} = 500\mu\text{s}$, $f=50\text{Hz}$)



(b) Liberation quantity from two-layer films (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) after 10 potential pulses stimulation ($E_{\text{red}} = -0.45\text{V}$, $E_{\text{ox}} = 0.3\text{V}$, $t_{\text{red}} = t_{\text{inter}} = 200\mu\text{s}$, $t_{\text{ox}} = 500\mu\text{s}$, $f=50\text{Hz}$) as a function of time.

Figure 4.24: Liberation over time by potential stimulation of two-layers polypyrrole films.

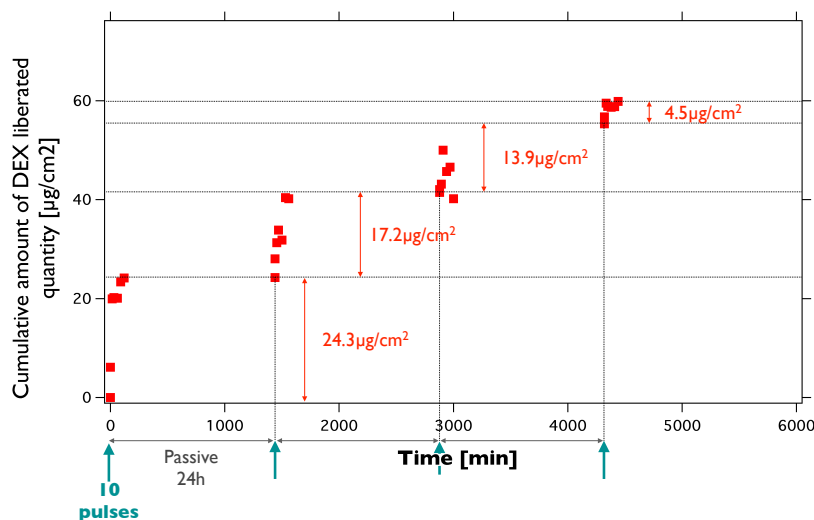


Figure 4.25: [Liberation quantity from a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$; PPy/DEX: $100\text{mC}/\text{cm}^2$) after 10 potential pulses stimulation series ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=50\text{Hz}$) as a function of time (one pulse series is applied per day).

4.3.4.2.B.4 Active liberation: in-vivo liberation In the aim of developing a long-term implanted electrical stimulation electrode, we firstly focused on the in vitro parameters to be optimized for the best control on the liberated quantity, as described in the previous parts of this chapter. Because of the highly simplified medium used in the in vitro experiments, among other parameters, the drawn conclusions cannot directly be applied to an in vivo behaviour of dexamethasone liberation from polypyrrole by biphasic potential pulses. In this work, a first series of in vivo experiments were conducted on a rat model. The parameters for the liberation were fixed as presented in the material and method section, as the reference situation to ensure on one hand the liberation of dexamethasone and on the other hand the stability in the functioning of the stimulation device.

Figures 4.26 show the site of implantation for cuff electrodes on a rat sciatic nerve, as accomplished in the MOEX lab. The left leg is implanted with a cuff electrode covered or not with polypyrrole films and directly stimulated with a single 1000 pulse series under anesthesia. After one day, the animals were sacrificed to analyze the morphology of the nerves. Right leg sciatic nerve were used as references. Figure 4.27 shows the histological morphology of transversal cut on rat sciatic nerves in function of the characteristics of the electrodes⁵.

First, we can observe on figures 4.27.(A) and 4.27.(B) respectively histological pictures of the

⁵These pictures are representative of the mean results obtained after the quantification and observation of the all animals and the corresponding references. Quantifications were done in the MOEX lab by Anne-Catherine Gerard. These results are presented as a introduction to the future in vivo work.

Chapter 4. Dexamethasone electrically controlled release from polypyrrole-coated electrodes

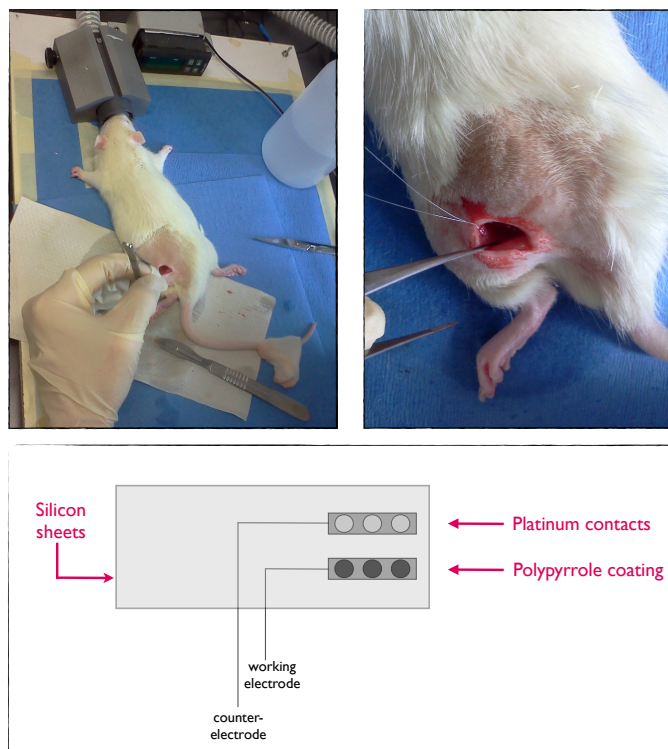


Figure 4.26: Pictures of rat sciatic nerve implantation of cuff electrodes before stimulation and the corresponding cuff design.

transversal cuts of a non-implanted nerve and an uncovered three-platinum contacts cuff electrode implanted nerve after one day, as previously described by Thil et al. [55]. We distinguish a thickening in the epineurium of the implanted nerve, attributed to the surgical procedure and the presence of the cuff electrode (no stimulation was applied). The thickness of the perineurium is also increased in the case of implanted nerves.

Figures 4.27.(C) and 4.27.(D) show the histological images of one day cuff implanted nerves with polypyrrole-coated platinum contacts (single PPy/Cl film and two-layer (PPy/Cl + PPy/DEX) film), after a 1000 pulses stimulation with automatic potential variation (see materials and methods for more informations). First, the implantation of a single polypyrrole film coated cuff electrode reveals a huge inflammatory reaction, with a significant thickening of both epineurium and perineurium. Moreover, while extracting the implanted nerve, we noticed an extensive inflammatory reaction outside of the cuff electrodes. This was attributed to the presence of the polypyrrole film, as it was observed even without electrical stimulation. It is of great importance in our case because literature reports numerous examples of polypyrrole-covered stimulation electrodes considered biocompatible [56–58]. The observed morphology changes on the nerves demonstrate a need for a detailed analysis of the characteristics of polypyrrole (dopant, morphology, stimulation, etc.) on the resulting inflammatory response.

We can observe on figures 4.27.(D).1 and (D).2 the morphology of a nerve after a one day implantation of a cuff electrode covered with a two-layers polypyrrole film (at different locations of the nerve). The liberation of dexamethasone was done using a single 1000 pulses series (characteristics will be described hereafter). The same morphological changes than in the case of bare platinum cuff can be observed. However, these changes are smaller than in the case of PPy/Cl electrodes. It is attributed to the anti-inflammatory activity of the liberated molecules, and thus corresponds to an indirect proof for the delivery of dexamethasone from the electrodes in an in-vivo environment. It is not possible at present to quantify precisely the amount of dexamethasone liberated in the surroundings of the cuff electrodes by the stimulation, nor the distance of action for those molecules in this configuration. It is nevertheless a proof of concept for the possibility to apply this liberation technique in an autonomous implanted device.

As a first approximation, the same electrode fabrication and liberation conditions were applied in-vitro (one series of 1000 pulses, $E_{red} = -0.45V$, $E_{ox} = 0.3V$, $t_{red} = t_{inter} = 200\mu s$, $t_{ox} = 500\mu s$, $f=50Hz$) in a PBS buffer. Electrodes were first kept 5 days immersed into a PBS solution to ensure the passive release of dexamethasone from the surface of the electrode (on the polypyrrole films or the silicon). The quantity of dexamethasone liberated after 1000 pulses and 2 hours of passive release is $\sim 250\mu g/cm^2$. This amount is higher than the ones measured on larger plane electrodes (of about one order of magnitude), even considering the bigger number of pulses used here. This needs to be investigated further on.

Previous research showed that dexamethasone is effective at a concentration of 0.2-0.7 μM [11]. Around this local concentration, a pronounced reduced inflammatory tissue reaction is observed around the neural implant. By approximation, a $0.5\mu g/cm^2$ of DEX release will result in an average DEX concentration of $1\mu M$ within $500\mu m$ radius from the electrode. The local concentration around the cuff electrode is thus $500\mu M$. This means that with a really small number of pulses (down to one or two pulses), the local delivery would be efficient to reduce the inflammation.

On figure 4.28 is shown the quantification for the thickness of the perineurium, epineurium and inflammation after a one day implantation of a non-coated cuff electrode (Ctrl), after a one day implantation of a cuff electrode recovered with a single PPy/Cl film ($50mC/cm^2$) and a 1000 pulses stimulation (PPY stim), and after a one day implantation of a cuff electrode recovered with a two-layer film (PPy/Cl: $50mC/cm^2$ + PPy/DEX $100mC/cm^2$) and a 1000 pulses stimulation (DEXA stim) ($E_{red} = -0.45V$, $E_{ox} = 0.3V$, $t_{red} = t_{inter} = 200\mu s$, $t_{ox} = 500\mu s$, $f=50Hz$). We can first conclude from this quantification that the inflammation is high on implanted and stimulated cuff electrodes without release of dexamethasone (single polypyrrole film). Furthermore, the inflammation is significantly reduced while in the presence (and the electrical stimulation) of an electrode with a polypyrrole film containing dexamethasone. This is thus an indirect proof of the delivery of anti-inflammatory active molecules in the direct surrounding of the nerve. More in-vivo investigations and quantifications need to be done to confirm these encouraging results.

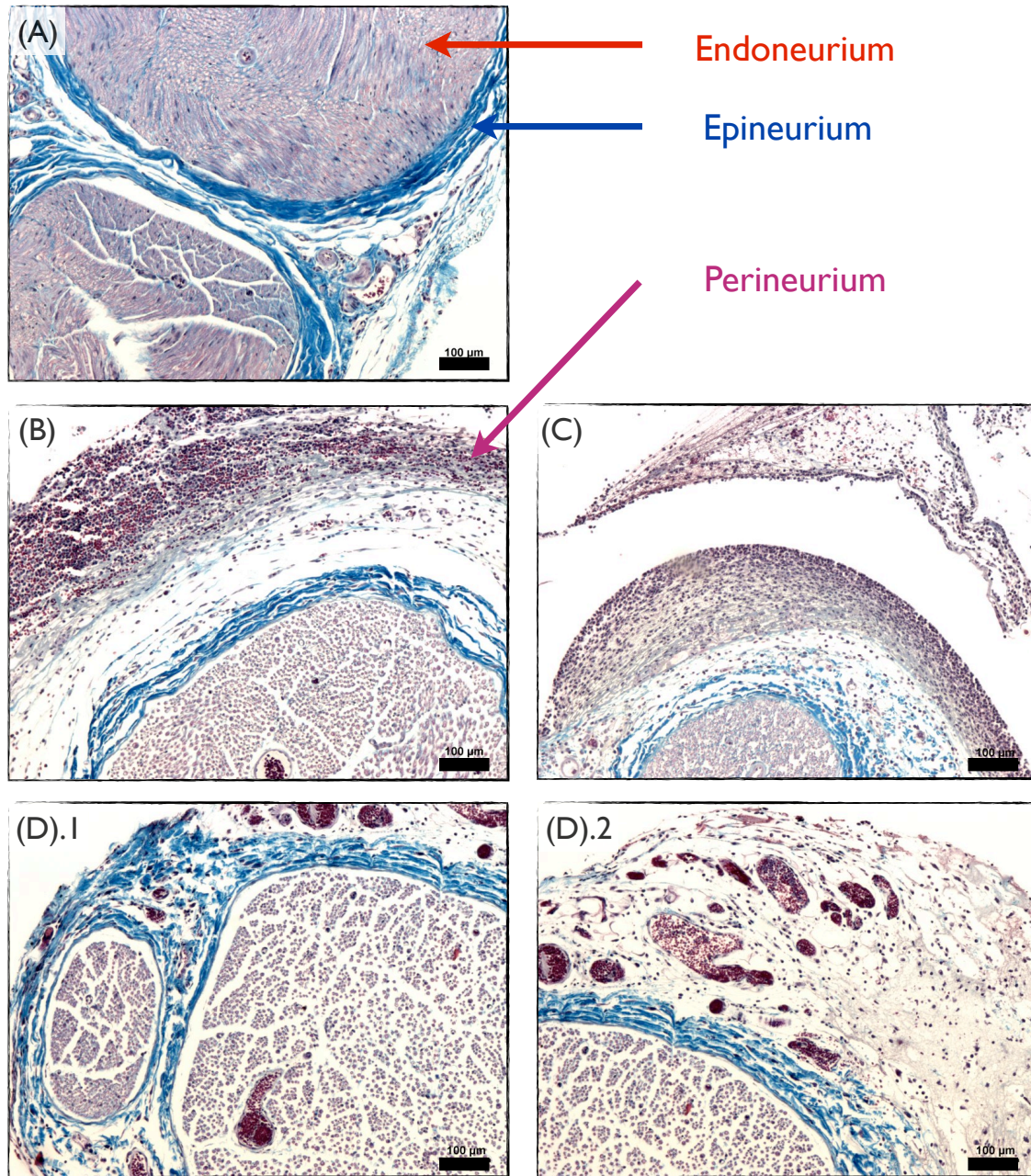


Figure 4.27: Histology pictures of transversal cuts on rat sciatic nerves (A) non-implanted (B) after a one day implantation of a cuff electrode with platinum contacts (C) after a one day implantation of a cuff electrode recovered with a single PPy/Cl film ($50\text{mC}/\text{cm}^2$) and a 1000 pulses stimulation ((D).1 and (D).2) after a one day implantation of a cuff electrode recovered with a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$ + PPy/DEX $100\text{mC}/\text{cm}^2$) and a 1000 pulses stimulation ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=50\text{Hz}$). Scale bar is $100\mu\text{m}$.

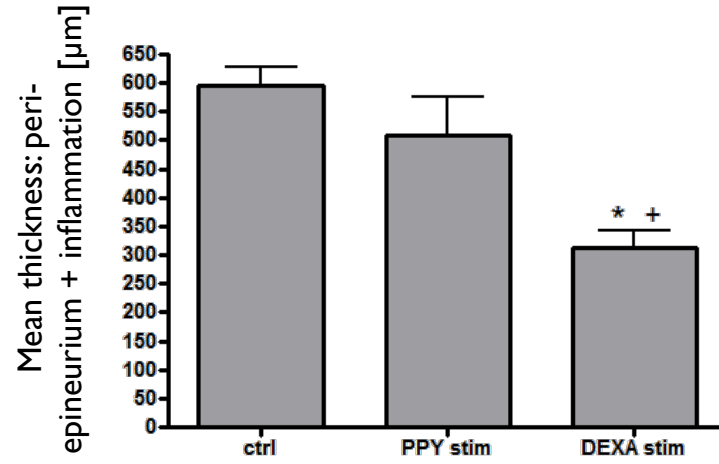


Figure 4.28: Mean thickness for the measurements of perineurium, epineurium and inflammation on rat sciatic nerves ($n=4$, ≥ 40 measurements per sample) after a one day implantation of a non-coated cuff electrode (Ctrl), after a one day implantation of a cuff electrode recovered with a single PPy/Cl film ($50\text{mC}/\text{cm}^2$) and a 1000 pulses stimulation (PPY stim), and after a one day implantation of a cuff electrode recovered with a two-layer film (PPy/Cl: $50\text{mC}/\text{cm}^2$ + PPy/DEX $100\text{mC}/\text{cm}^2$) and a 1000 pulses stimulation (DEXA stim) ($E_{red} = -0.45\text{V}$, $E_{ox} = 0.3\text{V}$, $t_{red} = t_{inter} = 200\mu\text{s}$, $t_{ox} = 500\mu\text{s}$, $f=50\text{Hz}$) [$p<0.05$, *vs Ctrl, + vs PPY stim].

4.4 Conclusion

In the first part of this chapter, we have developed a method allowing the easy and reproducible fabrication of switchable PPy-based nano-bioelectrodes for local controlled delivery of anti-inflammatory dexamethasone molecules. The drug incorporation was done using a one-step electropolymerization of a PPy film onto an electrode covered by a brush of metallic nanopillars. The release of the drug was carried out using cyclic voltammetry stimulus and was found to be electrically controllable. Due to the increased surface active area, the actuation redox potentials of nanostructured PPy were found to be lower than on conventional planar PPy films. Moreover, the presence of the Pt nanopillars onto the electrode substrates improves the mechanical stability and the adherence of the polymer onto the metallic substrate. Both of these parameters are of great importance for the development of long-term performances neural stimulation electrodes. The release was found to follow a first order quantitative release in function of the number of applied CV cycles. The influence of other technique-related parameters (scan rate, potential window) was also enlightened. However, the limits of the in-vivo use of cyclic voltammetry were revealed and at the same time reproducibility problems in the properties of the films.

We then described the optimization process of the fabrication technique to overcome these problems, based on a multi-layer approach. This synthesis technique, and furthermore the liberation properties of those two-layers polypyrrole films were analyzed by EQCM. A particular mixed behaviour in the charge compensation process was described.

Afterwards, a new technique for the release of molecules from these structures was developed, biphasic potential pulses. The concurrent development of the technical devices for the controlled application of this technique and the study of the important parameters ruling the quantitative liberation of dexamethasone from polypyrrole lead us to the isolation of a few parameters. The evolution of passive liberation in time following an active phase of stimulation was described, and a procedure to deliver small quantities of dexamethasone at a time was demonstrated.

Biphasic potential pulses stimulation was finally studied for the liberation of dexamethasone in-vivo, with the restrictions linked to the complexity of the biological environment.

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5 Electrosynthesis of single and multi-layer pyrrole 3-carboxylic acid copolymer films with tunable degree of functionalization for biomedical applications

This chapter deals with the electrosynthesis of thin copolymer films and with their outer surface modification with functional groups. At first, the synthesis of such copolymer films is investigated, together with the effect of three separate electrosynthesis parameters on the degree of functionalization. The second part of this chapter deals with the electrosynthesis of successive thin conducting polymer or copolymer films, and with their outer surface modification with functional groups. At first, the synthesis of such multi-layer structure is investigated. The consequences of the presence of an inner layer on the subsequent synthesis characteristics are analyzed. The possibility to deliver a molecule through this layer and the influence of this liberation on the functionalization are also examined by fluorescence microscopy.

The first part of this chapter is the subject of a publication in *Electrochimica Acta* [1].

5.1 Introduction

The third solution to improve electrode performances by surface modification considered in this work is the variation of the chemical composition. As already mentioned, conductive polymers combine the advantages associated to conventional polymers (low cost, simple fabrication process and wide choice of molecular structures) with electrical and optical properties similar to those of metals and inorganic semi-conductors [2–4]. Among the various conjugated polymers, polypyrrole (PPy) has gained tremendous significance due to its peculiar properties [5–7]. In spite of all these advantages, PPy suffers from the absence of any functional groups that can be used for biomolecules surface modification. As biologically tailoring the surface of biomaterials is highly desired to further enhance their performance in many biomedical applications, a lot of efforts have been done in recent years for elaborating functionalized PPy derivatives.

A common strategy for the functionalization of conducting polymers is the entrapment of biomolecules that acts as dopant during electrochemical polymerization. It has been demonstrated for different biomolecules [8–14] and shown an improvement of nerve cell adhesion and differentiation both in-vitro and in-vivo [8, 10, 11, 15]. The major problem of this procedure lies however in the control of the exact density or distribution of the biomolecules on the surface of the polymer, because the molecules are mainly present in the bulk of it. Non-covalent coupling techniques require a high concentration of molecules during the synthesis and may suffer from stability problem at the scale of time of tissue regeneration. Moreover, the generally demonstrated drawback is the detrimental influence on the mechanical and electrical properties.

Covalent tethering by either modification of the pyrrole monomers structure or through preparation of copolymers provides a highly explored alternative strategy for surface functionalization [8–13, 16–22]. Functionalized pyrrole units (ex. amino, carboxylic acid, active ester) have been used to specifically couple chemical compounds, peptides and proteins [19]. However, changes in the pyrrole structure often result in drops of electrical conductivity. For applications requiring good electroactive properties of the film, copolymers are thus preferred to pure substituted pyrrole monomers due to the modification of the pyrrole ring which affects the conductivity. Lee et al. [8] reports on the successful elaboration of polypyrrole copolymers from different monomer compositions, with a good conductivity after covalent modification of their surface. However, the presented synthesis technique can lead to irreversible reactions on the polymer itself (or on a previously synthesized polymer present on the electrode). De giglio et al. [23–26] showed the possibility to obtain thin copolymer films from pyrrole-3-acetic acid and evaluated their biocompatibility by in-vitro tests. Even though functionalized PPy surfaces were successfully prepared, scarce investigations have been performed regarding the detailed electrochemical copolymer synthesis.

In the previous chapter, we exhibited the possibility to synthesize polypyrrole films containing

molecules of interest and release them in a controlled way by interacting through film or/and electrochemical parameters. The combination of an external layer of functionalized copolymer film with an inner layer containing biologically interesting molecules is thus the logical continuation of the analysis. In literature, investigations on multi-layer systems of polypyrrole films have been done and some interactions between those two types of surface modifications were pointed out. K pas et al. studied the implications of layer-by-layer electrodeposition from a solution of the same composition on the ion transport properties [27]. They showed that the multi-layer structure results in charge/mass transport characteristics that differ from the properties of a single-layer electrode of the similar total thickness, the interfacial and bulk polymer properties being affected. The most significant effect pointed out was the hindrance of interlayer ion transfer. Tamm et al. [28] proved that the coverage of an inner polypyrrole layer can be obtained at low effective thickness of another polypyrrole outer layer. They also demonstrated the interactions between the two layers of different compositions during redox processes. Finally, Massoumi et al. studied the controlled release of molecules from bilayer systems of different constituting polymers [29, 30]. They demonstrated the possibility to release dexamethasone from bilayer films, and showed the effect of the outer layer on the reduction of the amount of molecules liberated.

Although, the liberation of molecules through a functionalized film has not yet been addressed, because of the limitations induced by both surface modifications. Among them, we can raise the following interesting fact: the modification (or functionalization) of a conducting polymer film often goes along with a poor electrical conductivity [15, 23–26], due notably to the chemical modification process in itself or the way to obtain a polymer comprising carboxylic acid functions available for further grafting. This disadvantage is not consistent with a long-term implantation and electrical stimulation, as well as for the liberation properties of the inner drug-loaded polypyrrole film. In fact, the concomitant limitations induced by those two surface modifications (drug delivery and functionalization) force researchers to be innovative as well as cautious in the purpose of the concurrent improvements brought by the complex assembly on one electrode.

In this chapter, we investigated the possibility to tailor a metallic surface with specific chemical groups for their further use in a modification process. This is done by the electro-copolymerization in aqueous solution of pyrrole (Py) and pyrrole-3-carboxylic acid (Py-COOH) on flat gold surfaces. The study of the electro(co-)polymerization was performed in order to evaluate the possibility to control the integration of the COOH-modified monomer and their effect on the electroactivity of the film. More precisely, the effects of the composition of the initial comonomer solution, the applied potential and the charge density passed for film formation, on the final degree of functionalization of the copolymer films were studied by FT-IR and X-ray photoelectron spectroscopy. The copolymerization electrosynthesis process was then adapted for preparing functionalized PPy nano-containers, presenting potential applications in drug delivery. Specif-

Chapter 5. Electrosynthesis of single and multi-layer pyrrole 3-carboxylic acid copolymer films with tunable degree of functionalization for biomedical applications

ically, functionalized (Py-co-Py-COOH) copolymer nanotubes were synthesized through the template method. The carboxylic acid groups available on the outer part of the nanotubes were then used to covalently immobilize poly(ethylene glycol) chains so as to enhance the antifouling properties of these new promising delivery vehicles¹.

In a second time, we analyzed the possibility to elaborate bilayer systems which combine both surface modification strategies (chemical modification on the surface and drug delivery from the bulk of the film). The construction of the studied electrodes are as follows. It begins with the synthesis of a polypyrrole film containing model molecules to be released (with relatively low molar mass : p-toluenesulfonic acid). The synthesis of a second layer onto this polypyrrole film is then studied. The composition of this second layer is the same as the one used previously, consisting in the copolymerization of pure pyrrole and a monomer unit with carboxylic acid functions. The synthesis of this peculiar structure was studied in terms of the control on the thickness and doping ratio via electrochemical quartz crystal microbalance (EQCM). The aim of this study is to evaluate the electrochemical properties of such complex structures. In this purpose, the electroactivity of these constructions was studied to ensure on one hand the release of the entrapped molecules through the top copolymeric layer and on the other hand to guaranty the good electrical properties of these structures. These properties were evaluated with electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and EQCM. Finally, fluorescence microscopy analyses were achieved on biotinylated-PEG chains incubated in an aqueous solution of *streptavidin*^{FITC} to ensure the effective covalent grafting on these multi-layer structure and evaluate the effect of stimulation on these graftings.

¹The latter part will not be discussed in the focus of this thesis. More details can be found in the published paper [1].

5.2 Materials and methods

5.2.1 Materials

Pyrrole (Py), pyrrole-3-carboxylic acid (Py-COOH) and lithium chloride ($LiClO_4$) were purchased from Acros, N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) from Sigma-Aldrich. Biotin-tagged poly(ethylene glycol)-amine (683 Da, amino-PEG-biotin) was obtained from Polypure (Oslo, Norway) and FITC-labelled streptavidin (*streptavidin*^{FITC}) from AbD Serotec (Dusseldorf, Germany). Gold coated silicon wafers were obtained from Platypus Technologies (Madison, United-States) and were cleaned by UV ozone treatment (5 min) and ethanol (20 min) before use. Before use, pyrrole monomer was purified by passing it over a micropipette filled with neutral alumina until transparent. Other products were used as received.

5.2.2 Electrochemical polymerization of Py and Py-COOH

Electrochemical polymerization of unmodified regular pyrrole and Py-COOH was carried out potentiostatically in a one-compartment cell with a Pt counter electrode and an Ag/AgCl/sat.KCl reference electrode at various potentials from 0.7 to 1.5V until the desired charge density was reached. All the potentials cited in this paper were calibrated with respect to Ag/AgCl/sat.KCl. The aqueous monomer solutions containing 0.03M monomer and 0.1M $LiClO_4$ were purged with N_2 for 10 min to prevent oxidation of the monomer prior to polymerization. The composition of the comonomer solution is noted x/y where x and y represent the molar percentage of the pyrrole monomer and the pyrrole-3-carboxylic acid monomer in the solution, respectively. The initial pH of the comonomer solution was about 5.5 and decreases throughout the polymerization process. Polymer films were synthesized on Au coated silicon surface (active area = 0.46cm^2) and the polymer film thickness was calculated from the polymerization charge using the conversion factor of 0.4Ccm^{-2} for $1\mu\text{m}$, reported by Pyo et al. for PPy/ ClO_4 films [31]. PPy/pTS films synthesis were performed in 0.1M Py + 0.025M NapTS aqueous solutions at 0.8V until a charge density of $100\text{mC}/\text{cm}^2$ (first layer). Copolymer films synthesis were carried out in 0.015M Py + 0.015M Py-COOH + 0.1M $LiClO_4$ aqueous solutions at 0.7V until a charge density of $50\text{mC}/\text{cm}^2$ (either on bare gold surface (single layer) or onto a first layer of polypyrrole synthesized as previously described (bilayer)) (see figure 5.1).

5.2.3 Characterization of Py/Py-COOH copolymer films

Polymer films were characterized by Fourier-transform infra-red spectroscopy (FT-IR) using a Nicolet Nexus 870 spectrometer coupled with a continuum Thermo Spectra-Tech microscope. Polymer films were deposited on a thallium bromoiodide crystal ($KRs - 5$) by scraping the surface of interest with a household aluminium foil. FT-IR spectra were taken at room temperature

Chapter 5. Electrosynthesis of single and multi-layer pyrrole 3-carboxylic acid copolymer films with tunable degree of functionalization for biomedical applications

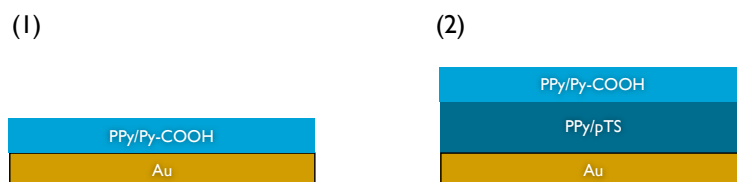


Figure 5.1: Diagram of the polypyrrole-coated samples: single PPy/Py-COOH layer on a gold substrate (1) and two-layer polypyrrole film (PPy/pTS + PPy/Py-COOH) on a flat gold surface (2).

in the transmittance range of $600\text{--}2000\text{cm}^{-1}$. Typically, 128 scans per spectrum were recorded at 2cm^{-1} resolution and at least three zones were investigated for each sample. To allow comparisons between the samples, a baseline containing the points at 1800 , 817 and 985cm^{-1} was drawn and the peak at 1545cm^{-1} (corresponding to the pyrrole ring stretching vibration [21]) was defined as the reference peak with its intensity equal to the unity. X-ray photoelectron spectroscopy (XPS) data were obtained using a SSI X-Probe (SSX-100/206) spectrometer from *Fisons*, equipped with a monochromatic Al $K\alpha$ X-ray source powered at 10 kV. The pressure in the analysis chamber was always maintained below 1.5×10^{-6} Pa. The energy resolution was set to 1.4 eV and the photoelectron take-off angle was 55° . The binding energy scale was set by fixing the C_{1s} component due to carbon only bound to carbon and hydrogen at 284.8 eV. The data treatment was performed with the *Casa XPS* program (Casa Software Ltd., UK). Peaks were decomposed using a linear baseline, and a component shape defined by the product of a Gauss and Lorentz function, in the 70:30 ratio, respectively.

5.2.4 Analysis of redox properties by EQCM

Polymer films were synthesized on Au coated quartz crystal surfaces (active area = 0.2cm^2) from *ChInstruments* using a *ChInstruments Model 400B* potentiostat with Time-Resolved Electrochemical Quartz Crystal Microbalance (EQCM). EQCM analysis of polypyrrole and copolymer films were carried out by cyclic voltammetry in a one-compartment teflon cell with a Pt wire as counter electrode and an *Ag/AgCl/sat.KCl* reference electrode using a *ChInstruments Model 400B* potentiostat with Time-Resolved Electrochemical Quartz Crystal Microbalance. Quartz crystals have a fundamental frequency of ~ 7.995 MHz and are coated with thin gold films on both sides. The gold disk deposited on the top side of the crystal is in contact with the electrolyte solution and used as the working electrode ($A=0.2\text{cm}^2$). CV experiments were done between +0.5V and -0.5V at a scan rate of 100 mV/s, after the application of a constant potential of 0.5V for 10s, either in monomer-free solution or in 0.1M NaCl in ultra pure water. The density of the quartz (ρ_q) is 2.684g.cm^{-3} and the shear modulus of the quartz (μ_q) is $2.947 \times 10^{11}\text{g.cm}^{-1}\text{s}^{-2}$.

5.2.5 Surface modification with poly(ethylene glycol)

The functionalization of the polymer surface with PEG chains was performed as follows: after electropolymerization, the samples were dipped in an aqueous solution containing 8mM EDC and 5mM NHS for 30min at ambient temperature to activate the carboxylic acid groups on the surface in an ester-activated form of N-sulfosuccinimidyl ester PPy. Then, amino-ended biotinylated-PEG chains (6 mM in aqueous solution) were chemically conjugated with the surface for 2h, leading to (biotinylated PEG)-grafted PPy-COOH surfaces. To validate this coupling reaction by fluorescence images, the samples were finally incubated in an aqueous solution of *streptavidin*^{FITC} ($50 \mu\text{g} \cdot \text{mL}^{-1}$) for 2h. Between each immersion, the samples were rinsed intensively with de-ionised water to remove the physisorbed compounds. Fluorescence images were obtained with a *LEICA* microscope equipped with a FITC filter set and an Epi-Fluorescence module, and connected to a *Hamamatsu* image-capture system. Images were processed using *IGOR Pro* software (*Wavemetrics, Inc.*). A silicium wafer placed on the polymer sample was used as background instead of the gold surface that reflects strongly the emission light. For bilayer polypyrrole films, the same grafting procedure was done, except for the samples stimulated by cyclic voltammetry. In this case, the same grafting procedure was done until the obtention of (biotinylated PEG)-grafted PPy-COOH surfaces. A cyclic voltammetry stimulation was then applied, for 50 cycles between +0.5V and -0.5V at a scan rate of 100mV/s, in an aqueous solution of 0.1M NaCl. The samples were finally incubated in an aqueous solution of *streptavidin*^{FITC} ($50 \mu\text{g} \cdot \text{mL}^{-1}$) for 2 h. All fluorescence images were obtained with a *LEICA* microscope equipped with a FITC filter set and an Epi-Fluorescence module using the same capture parameters (exposure time = 1s, gain = 1.5, resolution = 1392*1040). Images of bilayer polypyrrole films were processed using *ImageJ* image analysis software (version 1.45i), on 3 random areas of 3 samples. The amount of released pTS was quantified by UV absorbance at 220nm using a *Cary 500 scan* UV-vis-NIR.

5.2.6 Electrochemical Impedance Spectroscopy analysis

Impedance characterizations of gold flat surfaces and polypyrrole were performed using a *ChInstruments Chi660B* potentiostat in the same three-electrode cell arrangement as for synthesis. An *Ag/AgCl/3M NaCl* electrode (*Bio-Logic*) and a spring-like Pt wire (23cm, *Bio-Logic*) were used as RE and CE, respectively. The amplitude of the ac voltage was 5mV. Measurements were done at open circuit potential and 0.6V after applying the potential for 10s. The data measured in 0.1M NaCl were analyzed with the software *ZView* (Version 3.3a, *Scribner Associates Inc.*).

5.3 Results and discussion: electrosynthesis of pyrrole-3-carboxylic acid copolymer films with tunable degree of functionalization

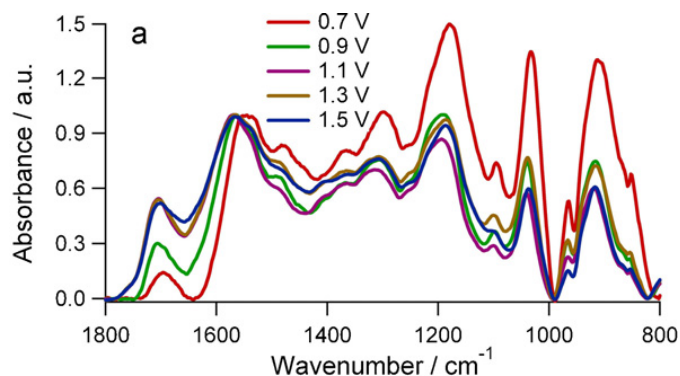
5.3.1 Synthesis and characterization of the polymer films

It has already been proved that electrochemical polymerization conditions have a huge impact on the morphology, as well as on the electrical properties of PPy and PPy derivative materials [6]. In this study, some of these electrosynthesis parameters, namely the applied potential, the composition of the monomer solution and the charge density passed for the film formation, which is correlated to the thickness of the film, have been investigated in order to evaluate their impact on the incorporation of carboxylic acid pyrrole comonomer into electrosynthesized copolymer films.

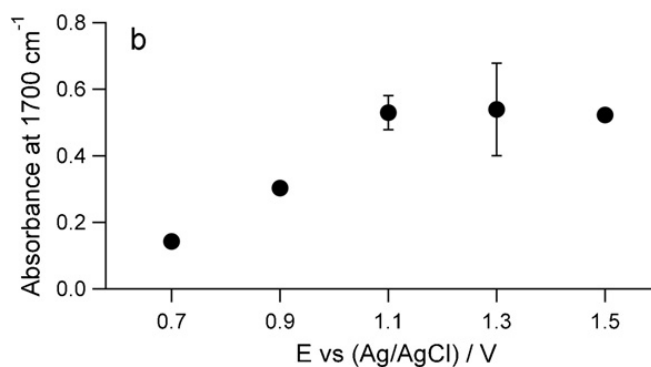
5.3.1.1 Effect of applied potential on PPy structure

Irreversible overoxidation of PPy occurs at potentials higher than $\sim 0.5\text{V}$ versus Ag/AgCl leading to the formation of functional carbonyl groups in the polymer backbone. In order to be able to discriminate between the appearance of carbonyl groups due to PPy overoxidation and the presence of carboxylic acid groups coming from the incorporation of Py-COOH into the polymer film, we therefore firstly analyzed by FT-IR the structure of PPy films electrosynthesized at different applied potentials (see figure 5.2). In all FT-IR spectra presented in figure 5.2, the characteristic absorption bands of PPy [32–34] are observed: 1545cm^{-1} (Py ring stretching vibration), 1476cm^{-1} (Py ring stretching and C-H in-plane stretching vibration), 1319cm^{-1} (C-N in-plane deformation), 1201cm^{-1} (Py ring vibration), 1049cm^{-1} (Py ring C-H deformation vibration), 967cm^{-1} (C-C out of plane ring deformation vibration). In addition, a peak at $\sim 1720\text{cm}^{-1}$, attributed to carbonyl groups ($\text{C}=\text{O}$) at the β position of pyrrole ring related to the overoxidation of PPy during the polymerization reaction [35, 36] is also present in all spectra. Figure 5.2.b depicted the evolution of the absorbance intensity of this peak (related to the reference peak at 1545cm^{-1}) in function of the applied potential. The plot clearly shows that the formation of carbonyl groups, and thus overoxidation of the polymer chains, increases with the oxidation potential applied during the electropolymerization process. As the carbonyl peak overlaps with the peak associated to the symmetric $\text{C}=\text{O}$ stretching vibration of carboxylic acid groups, appearing at $\sim 1700\text{cm}^{-1}$ [12, 33, 37], the contribution of the overoxidation peak will be taken into account in all further studies on Py and Py-COOH copolymerization. It should be emphasized that, as overoxidation is associated with irreversible structural changes and, consequently with a strong decrease of the electrical conductivity of PPy film, limiting as far as possible this degradation process is highly desired for many applications requiring conducting polymer films.

5.3. Results and discussion: electrosynthesis of pyrrole-3-carboxylic acid copolymer films with tunable degree of functionalization



(a) FT-IR spectra of polypyrrole films synthesized at different applied potentials.



(b) Plot of the absorbance intensity of the peak at 1700cm^{-1} versus the applied potential for polypyrrole films. The peak at 1545cm^{-1} is defined as the reference peak, *ie* its intensity equals the unity.

Figure 5.2: FTIR analysis of the absorbance of polypyrrole films as a function of the applied electrosynthesis potential.

5.3.1.2 Effect of the applied potential on the incorporation of Py-COOH

Copolymer films of same thickness were electrosynthesized from a 50/50 Py/Py-COOH comonomer solution at different applied potentials and the impact of the latter parameter on the degree of incorporation of Py-COOH units in the film was studied by FT-IR. As for pure PPy films, the results are expressed in terms of the absorbance intensity at 1700cm^{-1} with respect to the one at 1545cm^{-1} versus the oxidation potential applied during the electropolymerization (figure 5.3). The influence of overoxidation has been taken into account by subtracting the absorption values obtained for pure PPy from those obtained for copolymer films. As shown in figure 5.3,

Chapter 5. Electrosynthesis of single and multi-layer pyrrole 3-carboxylic acid copolymer films with tunable degree of functionalization for biomedical applications

the relative absorbance intensity decreases when the applied potential increases, indicating that a larger content of Py-COOH units is incorporated in the copolymer at lower potential. The possible electropolymerization of Py-COOH even at low oxidation potential, such as 0.7V, is consistent with previous studies reporting that the oxidation potential of pyrrole-3-carboxylic acid is comparable to the one of pyrrole monomer [24]. At higher potentials, the plot presents negative values for the absorbance intensity after PPy overoxidation correction. This seems to indicate that the polymer film undergoes less overoxidation when grown from a solution containing Py-COOH. This behaviour could be explained by the incorporation of functionalized comonomers mainly at the beginning of the electrosynthesis creating a *passivating* layer, PPy-COOH being less conductive than PPy [13]. The system can indeed be schematically represented by an electrical circuit composed of two resistances connected in series: the resistance of the copolymer film R_c and the resistance of the PPy film R_p (the resistance of the electrolyte solution is not taken into account for sake of simplicity). As the voltage across the two components is equal to the sum of the voltages across each component and, as $R_c > R_p$, the potential felt by the PPy film is consequently lower than the applied potential across the electrodes, leading to a decrease of the overoxidation of the polymer. However, the negative values of the relative absorbance at 1700cm^{-1} make the distinction between the incorporation of Py-COOH units into the copolymer and the overoxidation process difficult. The presence of carboxylic acid groups in films synthesized at high potentials ($\geq 1\text{ V}$) is therefore uncertain. Nevertheless, these experiments unambiguously demonstrate that the density of carboxylic acid functional groups in a (Py-co-Py-COOH) copolymer can be adjusted by tuning the applied potential and, that interestingly a higher density of carboxylic acid units is incorporated at low electrosynthesis potential.

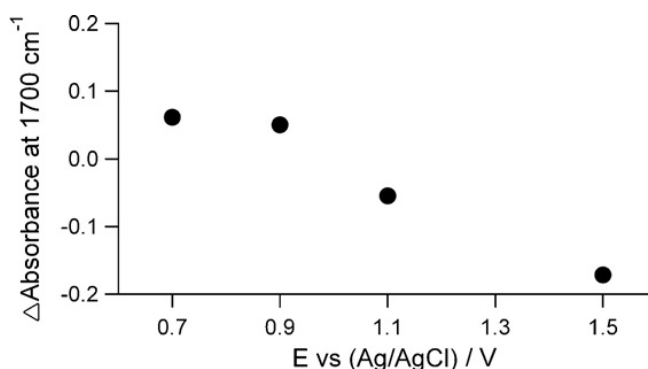


Figure 5.3: FTIR peaks analysis of the presence of caboxylic groups in polypyrrole films as a function of the synthesis potential: plot of the absorbance intensity of the peak at 1700cm^{-1} versus the applied potential for 50/50 copolymer films of 25 mC.cm^{-2} . The overoxidation contribution of the polypyrrole is subtracted. The peak at 1545cm^{-1} is defined as the reference peak, i.e. its intensity equals the unity.

5.3. Results and discussion: electrosynthesis of pyrrole-3-carboxylic acid copolymer films with tunable degree of functionalization

5.3.1.3 Effect of monomer solution composition on the incorporation of Py-COOH

In order to better control the proportion of incorporated modified pyrrole monomers (Py-COOH) in copolymer films, the influence of an other electrosynthesis parameter, the co-monomer solution composition, has been investigated. Previous studies performed with N-substituted pyrrole units (Py-N-COOH) reported on the influence of the composition of the comonomer solution on the resulting composition of the electrosynthesized film [12, 19]. These studies showed that increasing the molar percentage of Py-N-COOH in the comonomer solution leads to a higher content of Py-N-COOH units in the copolymer, but also that the electrical conductivity of the copolymer film severely decreases with the content of N-substituted pyrrole units, due to modifications of the structural planarity of π -electron conjugation. In this respect, the use of β -substituted pyrrole comonomer, as we did in this study, should present the advantage of significantly less affecting the electrical conductivity of the resulting copolymer film than N-substituted pyrrole [24, 38]. Py-co-Py-COOH copolymer films were synthesized at 0.7V (by passing a charge of 25 mC.cm^{-2}) from different comonomer solutions and characterized by FT-IR. Figure 5.4 presents the plot of the absorbance intensity at 1700 cm^{-1} versus the molar percentage of Py-COOH in the comonomer solution after overoxidation correction (the value corresponding to the overoxidation of PPy at 0.7V was subtracted from the obtained absorbance values for the copolymer films). At this electrosynthesis potential, it appears that the incorporation of Py-COOH units in the copolymer is strongly affected by the composition of the comonomer solution. The same trend than the one reported for the N-substituted pyrrole copolymers [12, 19] was observed, an increase of Py-COOH units in the copolymer when the molar percentage of Py-COOH is increased in the comonomer electrosynthesis solution.

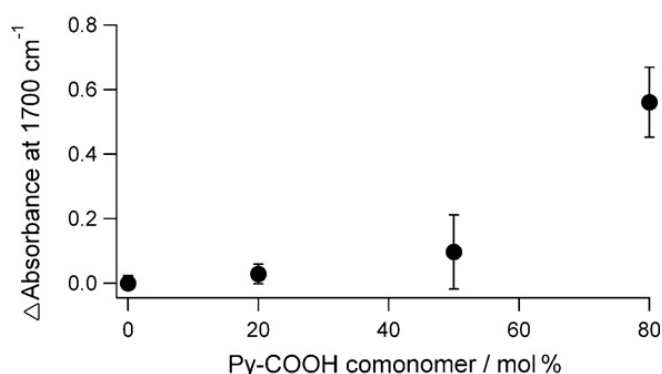


Figure 5.4: FTIR peaks analysis of the presence of caboxylic groups in polypyrrole films as a function of the comonomer solution: plot of the intensity of the peak at 1700 cm^{-1} versus the composition of the comonomer solution for polymer films of 25 mC.cm^{-2} synthesized at 0.7V versus (Ag/AgCl). The overoxidation contribution of the polypyrrole is subtracted. The peak at 1545 cm^{-1} is defined as the reference peak, *ie* its intensity equals the unity.

5.3.1.4 Effect of film thickness on the incorporation of Py-COOH

With copolymer film properties evolving with the film thickness [16], it is important to analyze the evolution of the composition of the copolymer film with its thickness. From a 50/50 comonomer solution, copolymer films were prepared at the potential of 0.7V with different charge densities ranging from 10 to 125 $mC.cm^{-2}$, corresponding to different film thicknesses[39]. Figure 5.5 presents the plot of the FT-IR absorbance intensity at $1700cm^{-1}$ versus the charge density passed for the formation of the film, without any overoxidation correction. These results show that the proportion of Py-COOH units incorporated into the copolymer decreases slightly when the film thickness increases. To further verify this observation, XPS

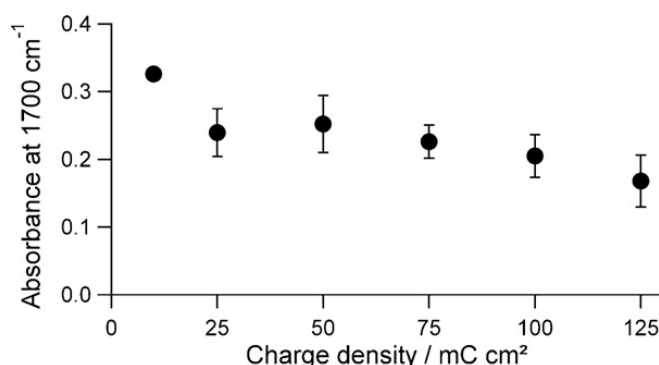


Figure 5.5: FTIR peaks analysis of the presence of carboxylic groups in polypyrrole films as a function of the synthesis charge: plot of the intensity of the peak at $1700cm^{-1}$ versus the charge density for 50/50 copolymer films synthesized at 0.7V versus (Ag/AgCl). The peak at $1545cm^{-1}$ is defined as the reference peak, i.e. its intensity equals the unity.

analyses were performed on 50/50 copolymer films prepared with a passed charge density of 25 and 100 $mC.cm^{-2}$ (corresponding to films of ~60 and 250 nm, respectively) and compared with pure PPy film of 25 $mC.cm^{-2}$. Survey scans showed the presence of C, N, O and Cl signals in all samples (data not shown), whereas no substrate signal was observed. C_{1s} peaks recorded on the different samples are presented in figure 5.6. Peak component assignments are based on previous studies [25, 40]. Details regarding the component position, peak area percentages and the corresponding assignments are reported in table 5.1. The differences observed in the C_{1s} peak components A and B, corresponding to C_{β} and C_{α} of the pyrrole ring, respectively, but also to adventitious contaminations, cannot be explained in a simple way. However, the variation of the contribution A with regard to B (figure 5.6) can not influence the interpretation of the evolution of components at higher binding energies, as the sum of A and B contributions do not appreciably vary for the different samples, in agreement with previous reported study [25]. The main difference between PPy (figure 5.6.a) and (Py-co-Py-COOH) copolymer (5.6.b and 5.6.c) surfaces is expected to be linked to the presence of COOH groups. Interestingly, the component at about 288.9 eV (component E, figure 5.6) attributed to carboxylic acid groups (C O)-O(H) [32,33] is significantly higher for the thinner copolymer ($Q = 25 mC.cm^{-2}$) than for both other

5.3. Results and discussion: electrosynthesis of pyrrole-3-carboxylic acid copolymer films with tunable degree of functionalization

samples, the thicker copolymer ($Q=100 \text{ mC.cm}^{-2}$) and PPy, that present a contribution of the same order (table 5.1). It should, however, be mentioned that in contrast to Py-co-Py-COOH copolymers, the contribution at 288.9 eV is attributed to a shake-up for pure PPy films [25, 38]. The XPS analyses thus prove that the amount of carboxylic acid groups at the surface is higher in the thinner copolymer film (film synthesized with a charge density of 25 mC.cm^{-2}).

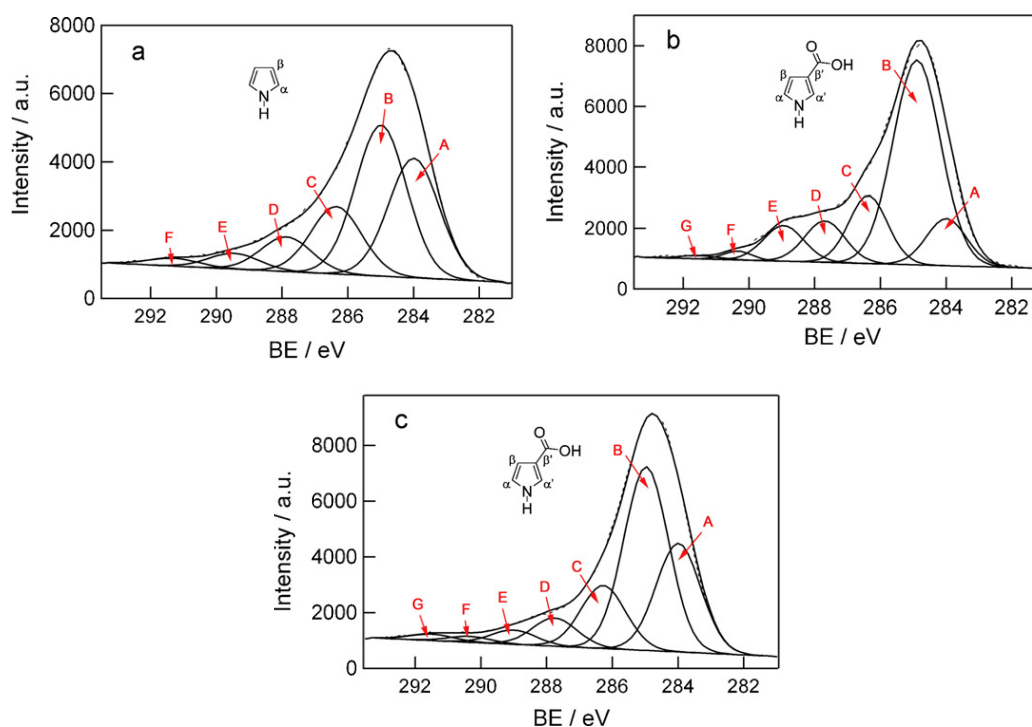


Figure 5.6: C_{1s} XPS peak recorded on (a) 25 mC.cm^{-2} polypyrrole film, (b) 25 mC.cm^{-2} and (c) 100 mC.cm^{-2} 50/50 copolymer films synthesized at 0.7V versus (Ag/AgCl). Curve fitting components (labelled peaks) and their resultants are also plotted. Assignment of peaks components are listed in Table 5.1.

To confirm this trend, the 50/50 copolymer films with the two different charge densities (namely, 25 mC.cm^{-2} and 100 mC.cm^{-2}) were reacted with amino-ended biotinylated PEG chains and further, incubated with *streptavidin*^{FITC} prior to characterization by fluorescence microscopy. A schematic of the different steps of the coupling reaction is presented in figure 5.7. Fluorescent optical microscopy images show a significantly higher green emission on the thinner copolymer film (figure 5.8.A, $Q = 25 \text{ mC.cm}^{-2}$) than on the thicker one (figure 5.8.B, $Q = 100 \text{ mC.cm}^{-2}$). These results, in good agreement with previous observations made by Dong et al. [13] using Py-N-COOH comonomer, confirm that Py-COOH units are preferentially incorporated in the early stage of the electrosynthesis which has an impact on the rest of the polymerization process in particular, on the overoxidation. This indicates that the content of carboxylic acid groups present at the surface of Py-co-Py-COOH copolymer films can also be tuned by adjusting the thickness of the electrosynthesized film.

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Table 5.1: Results of qualitative and quantitative XPS analysis for C_{1s} spectra of pure polypyrrole film with a charge density of 25mC.cm^{-2} and 50/50 copolymer films with charge densities of 25mC.cm^{-2} and 100mC.cm^{-2} .

	BE (eV)	Ppy (25mC.cm^{-2})		Copolymer (25mC.cm^{-2})		Copolymer (100mC.cm^{-2})	
		Area (%)	Assignment	Area (%)	Assignment	Area (%)	Assignment
C_{1s} A	283.9	30.11	C_β	10.67	$C_\beta, C_{\beta'}$	26.5	$C_\beta, C_{\beta'}$
C_{1s} B	284.8 (ref.)	38.16	C_α , cont.	54.93	$C_\alpha, C_{\alpha'}$, cont.	44.82	$C_\alpha, C_{\alpha'}$, cont.
C_{1s} C	286.3	17.03	$C-N^+$, $C-OH$, $C=N$	14.75	$C-N^+$, $C-OH$, $C=N$	15.38	$C-N^+$, $C-OH$, $C=N$
C_{1s} D	287.8	8.76	$C=O$	9.42	$C=O$	6.91	$C=O$
C_{1s} E	288.9	3.97	Shake-up	8.1	$(C=O)-OH$	3.5	$(C=O)-OH$
C_{1s} F	290.3	1.97	Shake-up	1.41	Shake-up	1.54	Shake-up
C_{1s} G	291.4	–	–	0.72	Shake-up	1.27	Shake-up

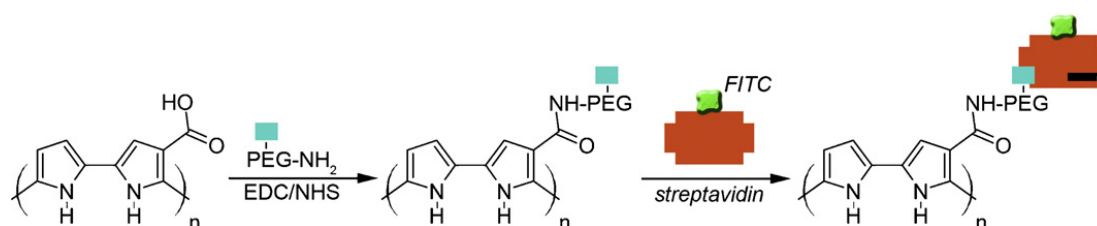


Figure 5.7: Schematic of the different steps of the chemical coupling reaction of carboxylic acid-functionalized polypyrrole covalently coupled with biotinylated amino-ended PEG via EDC/NHS and $streptavidin^{FITC}$.

5.4 Results and discussion: electrosynthesis of multi-layer copolymer films

5.4.1 Electrochemical synthesis and characterization of polypyrrole bilayers

5.4.1.1 Analysis of bilayers deposition by EQCM

Polypyrrole films were synthesized in the form of double layers (or bilayers) on gold coated EQCM quartz crystals. Figure 5.9 shows plots of the resonant frequency changes of the quartz resonators during the electrochemical synthesis of PPy/pTS and PPy-Py-COOH copolymer films as first and second layers respectively. The negative resonant shifts (Δf) with time (and charge) indicates a deposition of matter on the gold coating of the quartz crystal. Moreover, the relation between Δf and the charge passed (ΔQ) is linear, indicating an homogenous and linear film growth under the experimental conditions used in this work. The films obtained are dark coloured and homogenous.

PPy films synthesized by this chronoamperometric technique are in their oxidized form. In fact, two simultaneous reactions occur during synthesis : the backbone polymer formation (oxidation of Py monomer into oligomers, etc.) and the polymer oxidation. The latter is accompanied by anion incorporation, as charge compensation of polymer oxidation. The general expression for polypyrrole synthesis is thus equation 5.1 [41], where n is the degree of polymerization and a is

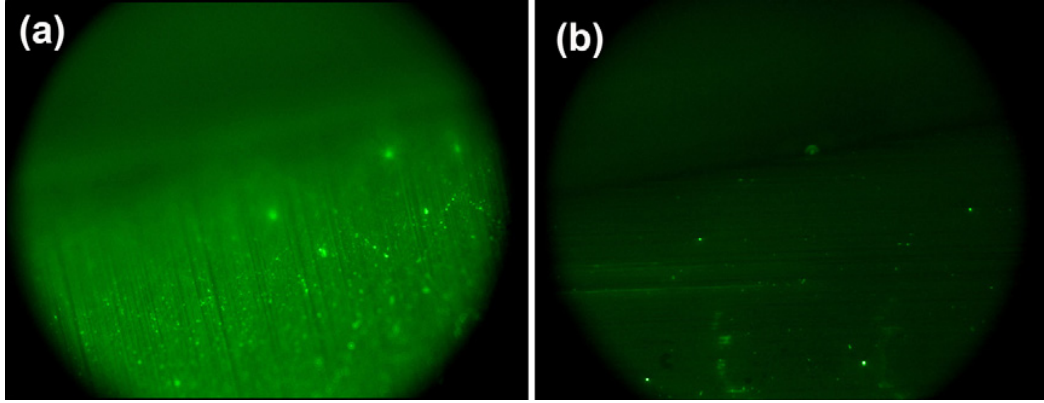
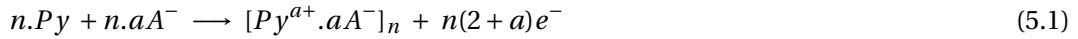


Figure 5.8: Fluorescent optical images of 50/50 copolymer film of (a) 25 mC.cm^{-2} and (b) 100 mC.cm^{-2} synthesized at 0.7 V versus (Ag/AgCl) after chemical coupling with amino-ended biotinylated PEG via EDC/NHS and incubation with *streptavidin*^{FTC}. The blurred part on the top of the images is due to the silicium wafer placed on the polymer sample and used as background.

the doping level (number of anions per pyrrole unit [42]).



Two different calculations techniques were used to estimate the relation between polypyrrole film thickness and the synthesis parameters. The first one (①) is based on the Sauerbrey relation and mass variations measured by EQCM. This first relation is represented by equation 5.2, where h is the thickness of the polypyrrole film synthesized on the gold coated quartz, ΔQ_s the charge per surface unit for the synthesis, V the volume of the polymer film, ρ its density and Δm_Q the mass variation induced by polypyrrole deposition per charge unit.

$$V = h.S = \frac{\Delta m}{\rho} \iff h = \frac{\Delta m_Q \cdot \Delta Q_s}{\rho} \quad (5.2)$$

A second way (②) to approximate the relation between charge and thickness of a dry polypyrrole film is reported in Akieh et al. [44], based on reported values of density (ρ) and apparent molar mass (M_{app}), where Q is the amount of charged passed during polymerization, n is the number of electrons involved in the polymerization and the degree of doping, A is the area of electrode and F is the Faraday's constant (equation 5.3). This latter is only based on the charge for synthesis in a first time (without considering EQCM measurements).

$$h = \frac{QM_{app}}{nFA\rho} \quad (5.3)$$

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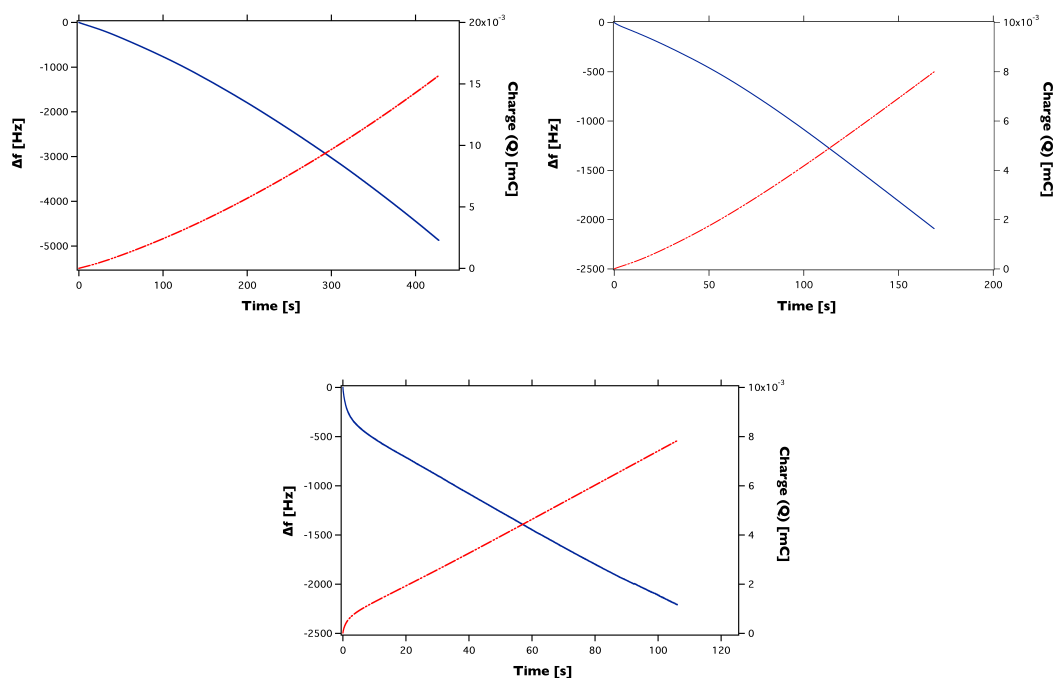


Figure 5.9: Frequency-time (—) and charge-time (---) plot for the growth of polypyrrole films (a) PPy/pTS film (0.8V *vs* Ag/AgCl; 0.1M Py + 0.025M NapTS) ; (b) Py-Py-3-COOH (50-50) copolymer film as single layer (0.7V *vs* Ag/AgCl; 0.015M Py + 0.015M Py-3-COOH + 0.1M LiClO₄) ; (c) Py-Py-3-COOH (50-50) copolymer film as outer layer of a PPy/pTS film (0.7V *vs* Ag/AgCl; 0.015M Py + 0.015M Py-3-COOH + 0.1M LiClO₄).

Finally, the thickness can also be observed by SEM, as a confirmation of the previous approximations.

Table 5.2 shows the parameters for approximation ① based on the EQCM measurements for the synthesis of PPy/pTS as first layer and copolymer films as first and second layers (on PPy/pTS). We can see that the relation for PPy/pTS is close to what was already observed in literature for similar conditions. For copolymer films, there are no data available in literature to compare to. Nevertheless, the relation seems realistic.

Table 5.2: Thickness *vs* charge relations for the synthesis of polypyrrole films (single and bilayers) using approximation ①.

Sample	Charge [mC/cm ²]	Time [s]	Δf [Hz/mC]	Δm [ng/mC]	Thickness <i>vs</i> charge
PPy/pTS	100	375.4 ± 116	- 305.7 ± 25	419 ± 34	43.3mC/cm ² for 100nm
Copo 1 st layer	50	173.9 ± 12	- 260.6 ± 70	340 ± 10	51.92mC/cm ² for 100nm
Copo 2 nd layer	50	100.6 ± 8	- 298.7 ± 56	409 ± 77	45.31mC/cm ² for 100nm

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The second approach (②) was done in three times. First (estimation #1), the apparent molar mass was considered as the one of pyrrole ($M_{app} = M_{Py}$) and n as the ideal case (2 1/3). In a second time (estimation #2), M_{app} was calculate while taking counterions into account (n is still the ideal value). Finally (estimation #3), the n value was evaluated from EQCM measurements and M_{app} re-evaluated based on this value. The obtained parameters for PPy/pTS films are shown in table 5.3.

Table 5.3: Thickness *vs* charge relations for the synthesis of PPy/pTS films using approximation ②.

Estimation of PPy/pTS synthesis parameters					Thickness <i>vs</i> charge
Estimation #	Charge [mC/cm ²]	ρ [g/cm ³]	n [-]	M_{app} [g/mol]	
1	100	1.48	2.33	67.09	60.5mC/cm ² for 100nm
2	100	1.48	2.33	121.55	33.5mC/cm ² for 100nm
3	100	1.48	2.106	83.2	44.3mC/cm ² for 100nm

For copolymers films, one more parameter has to be taken into account, to wit the molar mass of the monomer (see table 5.4). Actually, there are two monomers into the solution with different molar masses. Both monomer units will be inserted in a certain proportion into the polymer during electrochemical synthesis. At first approximation (estimation #1), the mean molar mass of the monomer was determined using the composition in the synthesis solution ($M_M = 0.5M_{Py} + 0.5M_{Py-3-COOH}$). The second approach (estimation #2) was applied in the same way as for PPy/pTS films. A n value was then calculated for the two types of copolymer films (single layer and outer layer of a PPy/pTS film) using those parameters, resulting in a negative value for the doping levels ($y = -0.53$ and -0.305 respectively), which is not possible. The assumption that M_M is composed of 50% of each monomer is thus not true. This will be discussed latter in this chapter, while the evaluation of the n number will be done using the redox exchange properties of polypyrrole.

Nevertheless, some interesting informations can be extracted from these data. We make an arbitrary hypothesis about the n value for the synthesis of copolymer films onto PPy/pTS (estimation #3), by assuming it the same as for the inner layer synthesis ($n=2.106$). This gives a M_{app} value of 81.3g/mol and thus the M_M value of 75.8g/mol. So, as $M_M = x_{Py} \cdot M_{Py} + (1 - x_{Py}) \cdot M_{Py-3-COOH}$, the value of $x_{Py} = 0.802$ was found. This means that one unit of Py-3-COOH is inserted every 5 monomeric unit in the backbone of the polymer film. This thus gives us clues to approximate the integration of modified pyrrole units into a polymer film, and to compare different situations with peculiar structures (single layer, bilayers, etc.).

Finally, we observed transversal cuts of PPy/pTS films by SEM to confirm the relations calculated. The relation gave us the value of $\sim 55 \text{ mC/cm}^{-2}$ for a 100nm thickness, which is close to the relations estimated using EQCM. By SEM, it was not possible to distinguish the layers in PPy/pTS

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Table 5.4: Thickness vs charge relations for the synthesis of copolymer films as single and outer layer of a PPy/pTS film using approximation ②.

Estimation of copolymer synthesis parameters (layer 1 or 2)					Thickness vs charge
Estimation #	Charge [mC/cm^2]	ρ [g/cm^3]	n [-]	M_{app} [g/mol]	
1	50	1.48	2.33	89,1	45.7mC/cm ² for 100nm
2	50	1.48	2.33	110.5	36.9mC/cm ² for 100nm
3 (layer 1)	50	1.48	1.47	-	-
3 (layer 2)	50	1.48	1.695	-	-
3' (layer 2)	50	1.48	2.106	81.3 ($M_M=75.8$)	45.3mC/cm ² for 100nm

and copolymer bilayers systems.

The present section proved that a copolymeric film can be synthesized onto either a bare gold electrode or on top of a PPy layer containing molecules of interest, and the possibility to control the thickness of the films. The impact of the presence of the second layer on the electroactivity of the inner layer can be analyzed, as well as the possibility to release pTS^- anions through the outer covering copolymeric layer.

5.4.1.2 Analysis of drug delivery from polypyrrole bilayers by EQCM

Ionic exchange properties of polypyrrole single and multi-layer films were investigated using EQCM. This study involves the description of each individual response on electrochemical redox reactions, leading to the previously described paths of ionic exchange. The general electrochemical reactions as well as the parameters influencing them were described in details in the state of the art of this thesis.

At first, as mentioned earlier in this chapter, Sauerbrey's approximation is considered valid for the synthesized structures (*ie* thin and rigid films). It was confirmed by the linearity of the mass deposited versus the electrical charge passed during synthesis [43]. As derived from the equations calculated in the previous section, the thicknesses of the different structures are : $\sim 225\text{nm}$ for PPy/pTS ($100\text{mC}/\text{cm}^2$) and $\sim 110\text{nm}$ (95nm) for the copolymer film as an second (single) layer ($50\text{mC}/\text{cm}^2$). An examination of the flux of ions (and solvent) during the potential cycling of PPy films was carried out and surveyed by EQCM. Potential scanings were done both in monomer-free synthesis solutions and NaCl 0.1M (as an oversimplified model of biological environment with small species carrying one charge).

Assuming that anions are expelled during reduction, the theoretical mass associated with their movement can be calculated from the electrical charge needed for this reaction. Based on Faraday's law, the charge required for the neutralization of the doped polypyrrole units is given

5.4. Results and discussion: electrosynthesis of multi-layer copolymer films

by: $Q_{A^-} = n_M F$; where n_M is the molar quantity of the anions involved in the charge movements. It can be written as: $Q_{A^-} = \frac{m_{A^-} F}{M_{A^-}}$; where m_{A^-} is the mass of anions A^- exchanged and M_{A^-} its molar mass. This is a theoretical value, assuming that all charges are used in the redox switching process. EQCM measurements provide the mass substituted from - or given to - the film. Combined with Sauerbrey's equation, it leads to relation 5.4, where $M_{app}^{A^-}$ is the apparent molar mass of species involved in the charge transport, ΔQ_{A^-} is the charge consumed during reaction and z the charge on the transferred ion.

$$\Delta m_{A^-} = \frac{M_{app}^{A^-} \Delta Q_{A^-}}{zF} \quad (5.4)$$

The plots of $\Delta m(EQCM)$ against Q are used to compute the apparent molar mass of the ions involved in charge compensation during the redox processes undergone by polypyrrole films in a particular electrolyte [44–46]. From these relations, $M_{app}^{A^-}$ are calculated and provide significant informations on the charge compensation processes involved in the studied system (dopant and electrolyte).

5.4.1.2.A EQCM analysis of PPy/pTS films Figure 5.10 shows the current and mass variations against the potential applied, using a scan rate of 100mV/s in a monomer-free solution of 0.025M NapTS in water, as described in the materials and methods section.

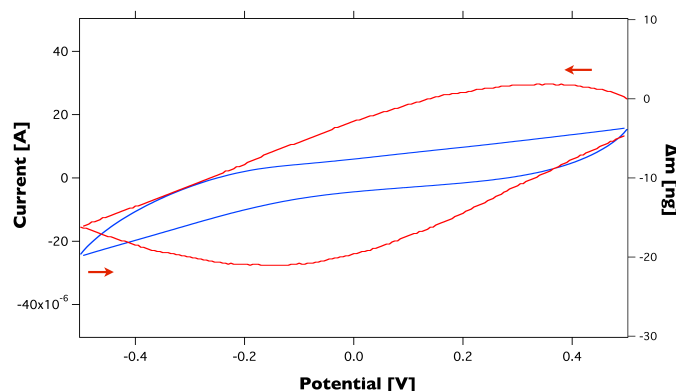


Figure 5.10: Second scan of current (—) and mass (—) variations accompanying oxidation and reduction of PPy/pTS films immersed in 0.025M NapTS. The potential range was from 0.5V to -0.5V, and the scan rate 100mVs^{-1} .

As observed, the electroactivity of the film in this solution is weak. At the beginning, the film is kept in its oxidized state (for 10s at 0.5V *vs* Ag/AgCl). In the reduction direction, we observe a small increase in mass until 0.3V and then a decrease until -0.5V. The latter can be attributed to pTS^- expulsion. In the oxidation direction, the film mass keep increasing until -0.2V and then the mass is partially regained. At the end of the cycle, the mass is smaller than its initial value for

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the oxidized film. This could be attributed to the impossibility to re-incorporate all pTS^- ions at this scan rate.

A mass diminution during reduction indicates a exchange path dominated by anionic movements. In literature, EQCM studies of polypyrrole films with pTS^- counter-anions in this exchange conditions conclude in a mixed ion exchange path [44].

Assuming that pTS^- anions are expelled during reduction, the theoretical mass associated with the movement of these species can be calculated from the electrical charge (see equation 5.4 and figure 5.11). On figure 5.11, we observe a contraction and a shift of the values of mass and charge. This is attributed to the evolution of the electroactivity of the film, as previously evoked, and the impossibility of full ionic re-incorporation over cycles. However, the theoretical mass associated with the movement of the species is not modified.

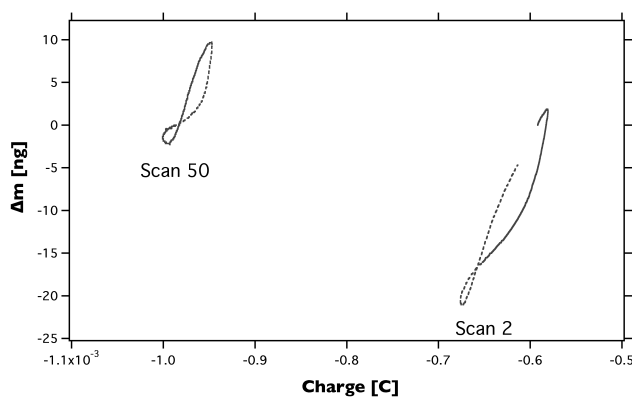


Figure 5.11: Mass changes as a function of charge during CV cycling (2nd and 50th scans, reduction (—) and oxidation (- - -)) of PPy/pTS in 0.025M NapTS.

From these values, the $M_{app}^{A^-}$ were calculated, and are gathered in table 5.5. Comparing the two directions of switching, we observe a difference in the observed apparent molar masses. If we consider that, during redox cycling, one mole of redox sites is converted and the electroneutrality is maintained strictly by the anions present in the electrolyte, the apparent molar mass calculated should be the one of pTS^- (171.1 g/mol). The calculated value is much lower. A possible explanation for this is that there are two simultaneous and competitive processes of the charge compensation (which understate the real molar mass), involving the transport of both anions (out of the film) and cations (into the film) during reduction (and inversely for oxidation). This is consistent with observations done by Naoi et al. [41, 47, 48].

If we consider the simultaneous movement of both anions and cations (in opposite directions), the apparent molar mass of the species involved into the charge compensation process (M_{app}^I) can be decomposed as:

$$M_{app}^I = \chi_{A^-} \cdot M_{app}^{A^-} - \chi_{C^+} \cdot M_{app}^{C^+} - \chi_S \cdot M_{app}^S \quad (5.5)$$

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Table 5.5: Apparent molar mass (M_{app}^{A-} [g/mol]) determined from the respective slopes during cathodic and anodic sweeping of PPy/pTS films immersed in aqueous 0.025M NapTS between 0.5V and -0.5V (100mV/s).

Ions	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (cathodic direction)	M_{app}^{A-} [g/mol]	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (anodic direction)	M_{app}^{A-} [g/mol]
$\text{Na}^+ ; \text{pTS}[0.025\text{M}]$	217	20.94	380	36.66

Note: the same signs for oxidation and reduction half-cycles reflect the reversal in direction of both mass and charge flow. When mass and charge have the same (opposite) direction, the dominant species involved in charge transport are anions (cations). A negative value for M_{app}^{A-} would correspond to a value of M_{app}^{C+} .

where χ_X is the corresponding proportion of the X species exchanged between the film and the electrolyte solution. We can mention here the concomitant movement of solvent molecules with cations (*ie* solvated cations) from and into the film².

Based on this affirmation, we can state that: $\chi_S = h_{C+} \cdot \chi_{C+}$; h_{C+} being the cation hydration number, depending on the nature of cations. For Na^+ cations, Annibaldi [43] et al. observed an hydration number of ~ 4 . As $\chi_{A-} = 1 - \chi_{C+}$, the solutions for this system are: $\chi_{C+} = 0.564(0.505)$ and $\chi_{A-} = 0.436(0.495)$ in the cathodic(anodic) direction. This reveals the mixed comportment of the charge compensation processes, with a higher proportion of the ion movement into the film than out of it. Still, the global exchange process as measured by EQCM is anionic.

Figure 5.12 shows the current and mass variations against the potential applied, using a scan rate of 100mV/s in a solution of 0.1M NaCl in water.

In this case, the same trend is observed: a decrease of mass during reduction. Quantitatively, we can notice a higher electroactivity. As we look at mass variation during the reduction phase of a single cycle, we see that mass decreases until $\sim -0.3\text{V}$ and then increases until -0.5V . The opposite response is observed in the oxidation direction, with the change in mass variation at $\sim -0.1\text{V}$. An evolution of mass variation behaviour can also be noticed. As CV goes along, the mass variation is modified, with an inversion of mass variation occurring at higher potentials, leading to a global increase of the mass of the film.

The plots of mass against charge can be seen in figure 5.13. Two regions of ion movement during redox cycling of the film in NaCl can be observed. As said previously, two competitive processes appear simultaneously, and this shows up on these plots, where the right part (❶) corresponds to the area where anion flux prevails and the left part (❷) where cation flux prevails in the charge compensation process. M_{app}^{A-} were calculated for this configuration, and can be observed in table 5.6.

Similarly to the previous system, the apparent molar masses of the ions involved in the charge

²Siritski et al. [41] considered that anion movement is also accompanied by solvent molecules in a smaller amount [41, 43, 44, 49]. As a simplification, we will not consider this situation in our work, as it is assumed in a lot of works reported in literature [44, 49], considering that the degree of hydration of anions incorporated into PPy films is too low to be detected by the EQCM measurements.

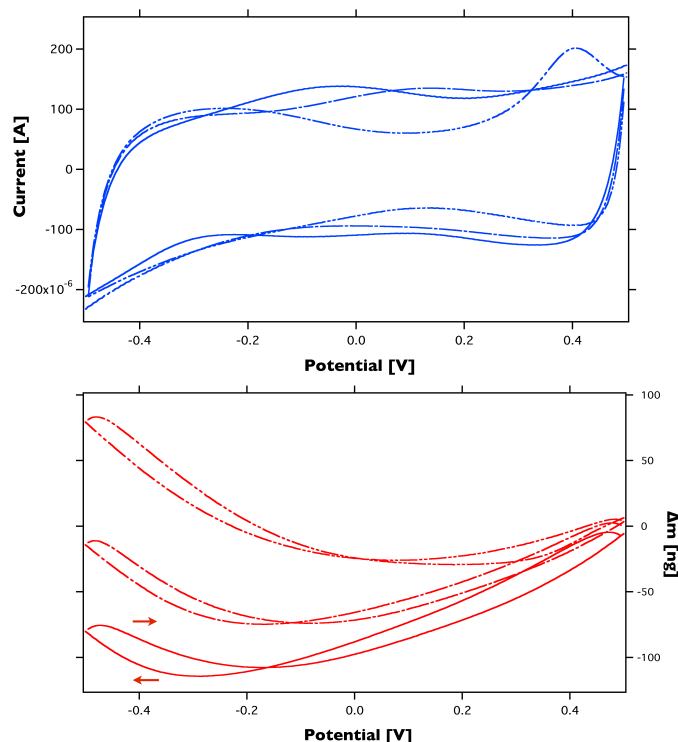


Figure 5.12: Scans of current and mass variations accompanying oxidation and reduction of PPy/pTS films immersed in 0.1M NaCl (scan number 2 (—), 12 (---) and 42 (- - -)). The potential range was from 0.5V to -0.5V, and the scan rate 100mV s^{-1} .

compensation process are not the ones of the ions in presence. Actually, the ❶ area should lead to an apparent molar mass of the pTS^- ion (171.1 g/mol) and the ❷ area to an apparent molar mass of Na^+ (23 g/mol) (if we consider non-solvated cations). The conclusion is the same as before, with a mixed compartment for the charge compensation process.

Moreover, the comportment evolves with the cycling of the film, showing a decrease in the apparent molar mass of the anion dominated region and the opposite in the cation dominated region. This is attributed to the variation of composition of the film. In fact, at each cycle, it is assumed that upon re-oxidation, a part of pTS^- anions is replaced by Cl^- from the electrolyte (because of the greater mobility of the small size anions inside of the polypyrrole film). At the next cycle, considering the lower molar mass of Cl^- anions, the global anionic mass exchanged per charge unit is smaller (if the ratio of anions to cations involved in the ionic movement remains the same). The corresponding apparent molar mass is thus logically smaller. The argument is the same for the increase of the apparent molar mass of the cationic dominated region.

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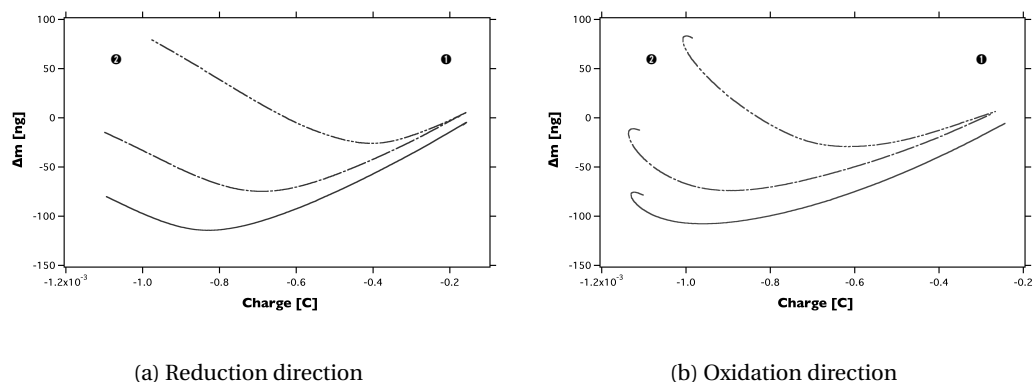


Figure 5.13: Mass changes as a function of charge during CV cycling (scan number 2 (—), 12 (---) and 52 (-.-)) of PPy/pTS in 0.1M NaCl.

Table 5.6: Apparent molar mass (M_{app}^{A-} [g/mol]) determined from the respective slopes during cathodic and anodic sweeping of PPy/pTS films immersed in aqueous 0.1M NaCl between 0.5V and -0.5V (100mV/s).

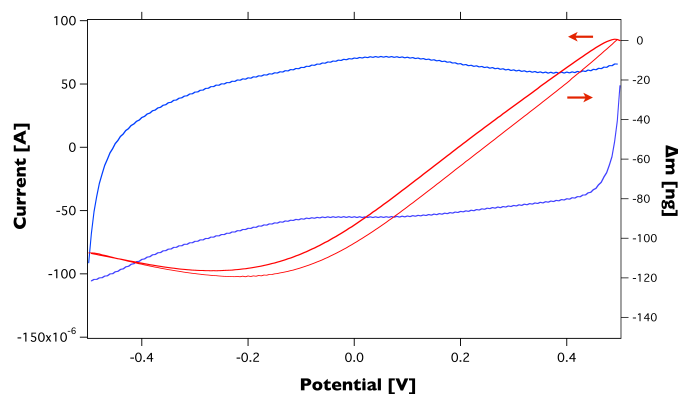
Ions	Cycle	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (cathodic direction)		M_{app}^{A-} [g/mol]		$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (anodic direction)		M_{app}^{A-} [g/mol]	
		1	2	1	2	1	2	1	2
$\text{Na}^+; \text{Cl}^-$ [0.1M]	2	198	-147	19.1	-14.2	173	-163	16.7	-15.7
	12	178	-174	17.2	-16.8	140	-266	13.5	-25.7
	42	148	-210	14.3	-20.3	120	-321	11.6	-31

Note: the same signs for oxidation and reduction half-cycles reflect the reversal in direction of both mass and charge flow. When mass and charge have the same (opposite) direction, the dominant species involved in charge transport are anions (cations). A negative value for M_{app}^{A-} corresponds to a value of M_{app}^{C+} .

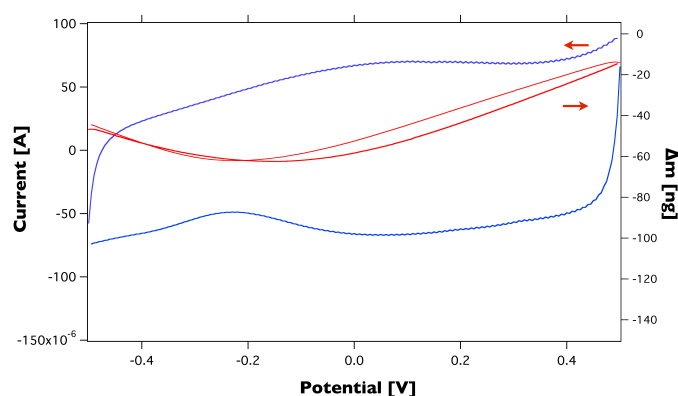
5.4.1.2.B EQCM analysis of copolymer films Figure 5.14 shows the current and mass variations against the potential applied to a copolymer film, using a scan rate of 100mV/s in a monomer-free aqueous solution of 0.1M LiClO_4 (a) and 0.1M NaCl (b).

At the beginning, the films were kept in their oxidized state (for 10s at 0.5V *vs* Ag/AgCl). In LiClO_4 , in the reduction direction, we observe a decrease in mass until $\sim -0.25\text{V}$ and then a light increase until -0.5V. The symmetrical opposite is observed when scanning in the oxidation direction. At the end of the cycle, the mass is the same as at the beginning. This is a typical behaviour for polypyrrole films with small univalent counterions in a monomer-free solution, as reported by literature [43]. In NaCl , the behaviour of the copolymer film is similar, except that a smaller mass variation is obtained.

The plots of mass against charge are reported in figure 5.15 for both electrolytes. In this case, two regions are also distinguished. Likewise for PPy/pTS films, these two regions correspond to the prevalence of ionic flux. Area 1 corresponds to the zone where anion flux prevails and area 2 where cation flux prevails. The respective values for M_{app}^{A-} were calculated, and can be



(a) $LiClO_4$ 0.1M



(b) $NaCl$ 0.1M

Figure 5.14: Second scan of current and mass variations accompanying oxidation and reduction of copolymer films immersed in different electrolytes. The potential range was from 0.5V to -0.5V, and the scan rate $100mVs^{-1}$.

observed in table 5.7.

We can distinguish two things of this table. First, the mass transport is mainly anionic up to a certain potential ($\sim -0.2V$) and then dominated by cations. The apparent molar masses are different in presence of the electrolytes, confirming the influence of the electrolyte on the liberation properties. In $LiClO_4$, the cationic part of the exchange corresponds to the molar mass of Li^+ , while the anionic dominated part of exchange shows a mixed comportment, with an apparent molar mass lower than the mass of the perchlorate ion. In $NaCl$, the behaviour is a little bit divergent about the quantitative conclusions that can be drawn. Both the apparent molar masses of anionic and cationic-controlled exchange transport do not reflect an extreme

5.4. Results and discussion: electrosynthesis of multi-layer copolymer films

single ionic exchange situation. All along the potential switching, a mixed behaviour is observed.

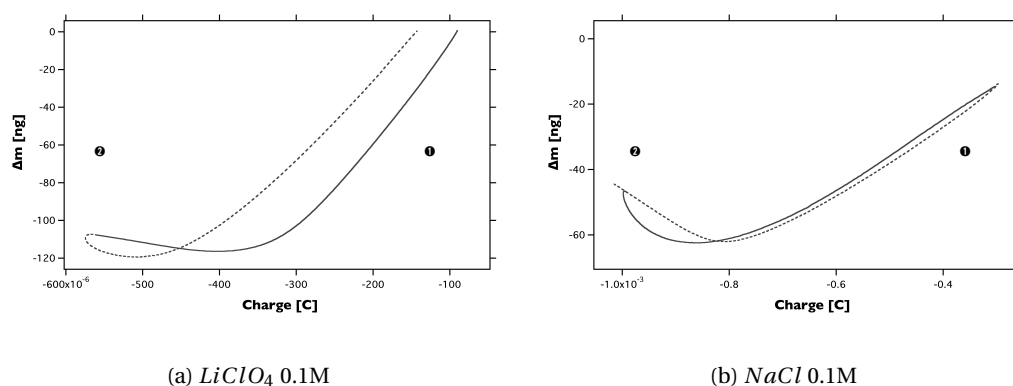


Figure 5.15: Mass changes as a function of charge during CV cycling (2nd scan, reduction (—) and oxidation (---)) of copolymer films in different electrolytes.

Table 5.7: Apparent molar mass (M_{app}^{A-} [g/mol]) determined from the respective slopes during cathodic and anodic sweeping of copolymer films immersed in aqueous 0.1M $LiClO_4$ or 0.1M $NaCl$ between 0.5V and -0.5V (100mV/s).

Ions	$\Delta m/\Delta Q$ [$\mu g/C$] (cathodic direction)		M_{app}^{A-} [g/mol]		$\Delta m/\Delta Q$ [$\mu g/C$] (anodic direction)		M_{app}^{A-} [g/mol]	
	①	②	①	②	①	②	①	②
$Li^+ ; ClO_4^-$ [0.1M]	504	-64	48.6	-6.2	416	-103	40.1	-9.9
$Na^+ ; Cl^-$ [0.1M]	103	-122	9.9	-11.8	100	-98	9.65	-9.5

Note: the same signs for oxidation and reduction half-cycles reflect the reversal in direction of both mass and charge flow. When mass and charge have the same (opposite) direction, the dominant species involved in charge transport are anions (cations). A negative value for M_{app}^{A-} corresponds to a value of M_{app}^{C+} .

5.4.1.2.C EQCM analysis of bilayer films Even though a lot of work has been done on the release properties from polypyrrole with different counter-ions, electrolytes, techniques for release and parameters of the film ; the bi-layer structure with proper counter-ions and release properties has not yet been explored. The isolate characteristics of each single film has been analyzed previously. This section presents the results of the same drug delivery properties exploration by EQCM, applied to the peculiar bi-layer structure of a PPy/pTS film covered with a copolymer polypyrrole film PPy/Py-3-COOH.

Figures 5.16 show the current and mass variations against the potential applied, using a scan rate of 100mV/s in a solution of 0.1M NaCl in water.

The electroactivity displayed is higher than the one of the single films. This will be discussed in more details in a next section. Mass variations present a shape similar to PPy/pTS films, with

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the same apparent evolution with the number of cycles. The amplitude, however, is higher for the mass variations, indicating an intricate ionic exchange, with a combination of the properties of both films.

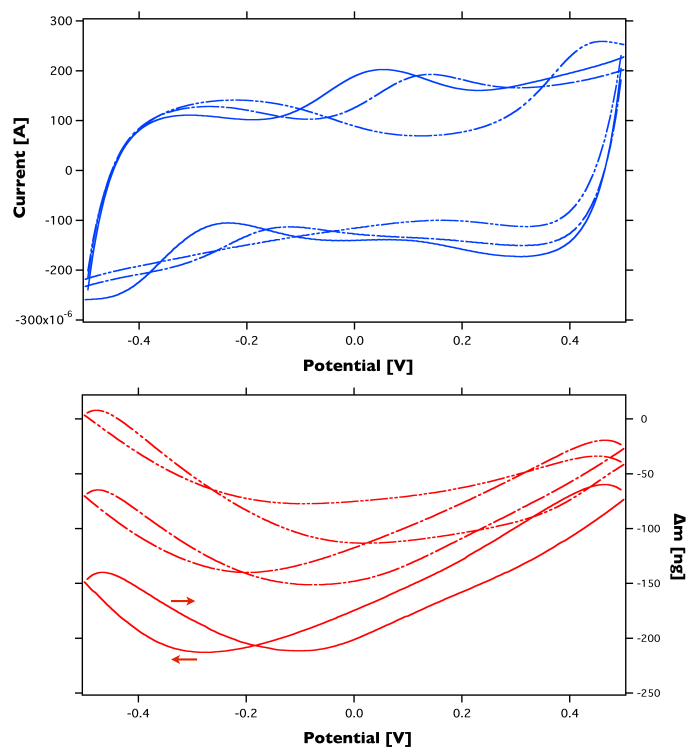


Figure 5.16: Scans of current and mass variations accompanying oxidation and reduction of bilayer films immersed in 0.1M NaCl (scan number 2 (—), 12 (---) and 42 (- - -)). The potential range was from 0.5V to -0.5V, and the scan rate 100mVs^{-1} .

The plots of mass against charge are reported in figures 5.17. Once again, the two regions can be identified corresponding to the same ion flux as before. The respective values for M_{app}^{A-} were calculated, and can be observed in table 5.8.

The apparent molar masses for bilayers systems appear to be close to the ones found for simple PPy/pTS films, with the same evolution over cycles. These values mean that the exchange to ensure the charge compensation process is a simultaneous mixed ionic species transport. The larger values for the mass moving in and out of the polypyrrole films are thus attributed to the concomitant exchange from both parts of the bilayer polypyrrole film. Furthermore, the domination of the mass compensation process can be attributed to the movement of pTS^- ions out of the film during the reduction phase. This is the first proof of the possibility to release a low mass anionic molecule from an inner polypyrrole film through a copolymer film.

5.4. Results and discussion: electrosynthesis of multi-layer copolymer films

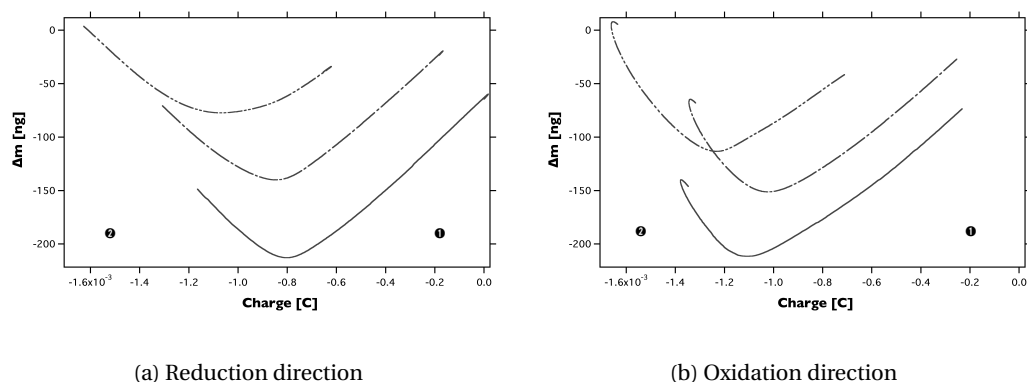


Figure 5.17: Mass changes as a function of charge during CV cycling (scan number 2 (—), 12 (---) and 42 (---)) of bilayers PPy films in 0.1M NaCl.

Table 5.8: Apparent molar mass (M_{app}^{A-} [g/mol]) determined from the respective slopes during cathodic and anodic sweeping of bilayer films immersed in aqueous 0.1M NaCl between 0.5V and -0.5V (100mV/s).

Ions	Cycle	$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (cathodic direction)		M_{app}^{A-} [g/mol]		$\Delta m/\Delta Q$ [$\mu\text{g}/\text{C}$] (anodic direction)		M_{app}^{A-} [g/mol]	
		①	②	①	②	①	②	①	②
$\text{Na}^+; \text{Cl}^-$ [0.1M]	2	208	-198	20.1	-19.1	170	-303	16.4	-29.2
	12	195	-170	18.8	-16.4	175	-310	16.9	-29.9
	42	137	-170	13.2	-16.4	150	-296	14.5	-28.5

Note: the same signs for oxidation and reduction half-cycles reflect the reversal in direction of both mass and charge flow. When mass and charge have the same (opposite) direction, the dominant species involved in charge transport are anions (cations). A negative value for M_{app}^{A-} corresponds to a value of M_{app}^{C+} .

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5.4.1.3 Electroactivity analysis by CV and EIS

In our case, the electroactivity is a very important parameter in the outlook of stimulation electrodes with long-term stable performances. It has been shown in the previous chapter the possibility to control the density of grafting on polypyrrole copolymer films. In the previous section, it was shown that the electroactivity of single copolymeric films is good.

To evaluate the electroactivity of each layer of bilayer film and the effect of covalent coupling on the electrochemical properties, CV and impedance measurements were done on the different layers. Figure 5.18 shows the electroactivity of the films measured by cyclic voltammetry in 0.1M NaCl at each step of the construction of a bilayer structure. The electroactivity of a single copolymer layer is also presented as a comparison. We first observe a significantly high capacitive current in the copolymer single layer, indicating a good electrical conductivity. Secondly, we see an increased current with the successive synthesis of PPy/pTS and copolymer as a second layer, compared to the flat gold electrode.

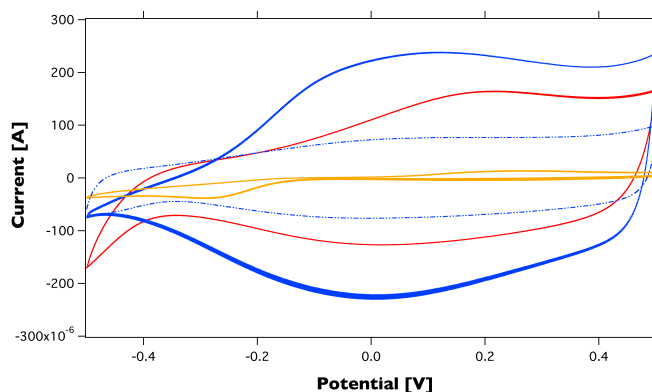


Figure 5.18: Current measurements during CV cyclings of gold (—) PPy/pTS (— ; $100\text{mC}/\text{cm}^2$), PPy/pTS + copolymer (—) and single copolymer (--- ; $50\text{mC}/\text{cm}^2$) films in 0.1M NaCl. The potential range was from 0.5V to -0.5V, and the scan rate 100mV s^{-1} .

EIS measurements on the same structures were done to evaluate the impacts on impedance of the synthesis of each layer separately and combined. Figures 5.19 and 5.20 show the module and phase angle for the EIS measurements on a single PPy/pTS film and on a bilayer electrode at two different potentials, compared to bare gold electrode. The first observation is that the influence of the potential applied (open circuit potential or 0.6V *vs* Ag/AgCl) is negligible. It can secondly be observed on this graph the reduction of impedance module engendered by polypyrrole films, either as a single or a bi-layer structure. This is a classical observation on conducting polymer films, where the charge transfer processes within the polymer dominate at high frequencies (while contributions to the impedance of the polymer come from mass transfer processes at the polymer-solution interface in the low frequency regime).

A comparison of impedance modules for the PPy/pTS films and bilayer structures shows that the impedance module is not significantly affected by the presence of a copolymeric film. This goes

5.4. Results and discussion: electrosynthesis of multi-layer copolymer films

in the opposite direction of results reported by literature on PPy (and/or copolymer) films with modified monomers [15], confirming the good conducting properties of the outer copolymer layer.

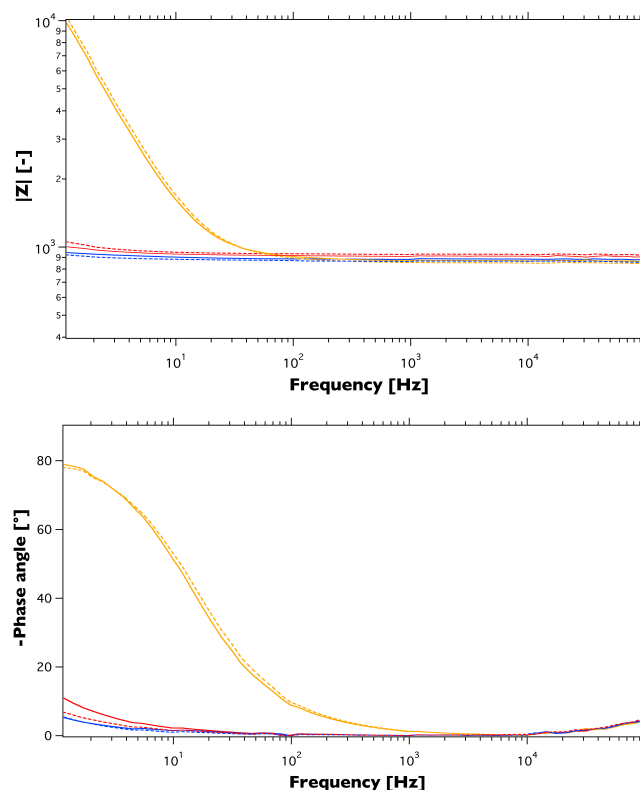


Figure 5.19: Complex plane bode plots of module and phase angle for bare gold (—) PPy/pTS (—; 100 mC/cm^2), PPy/pTS + copolymer (—; 50 mC/cm^2) films recorded in 0.1M NaCl using a perturbation amplitude of 5mV, measured at open circuit potential (—) and at a potential of +0.6V (---) vs Ag/AgCl.

EIS measurements were done on bilayer structures at each step of construction and after PEG grafting. Results are shown in figure 5.21. The trend is the same as the previous graph, except after PEG grafting on the outer copolymer layer. The impedance module at low frequency is increased by the chemical modification of the outer layer. The corresponding impedance phase angle is also modified. This phenomenon indicates an increase of the electron-transfer resistance at the electrode/electrolyte interface due to the increase number of molecules added to the electrode outer layer [50]. A study based on model equivalent circuits (modified Randles circuit) was done, but did not brought any supplementary quantitative information.

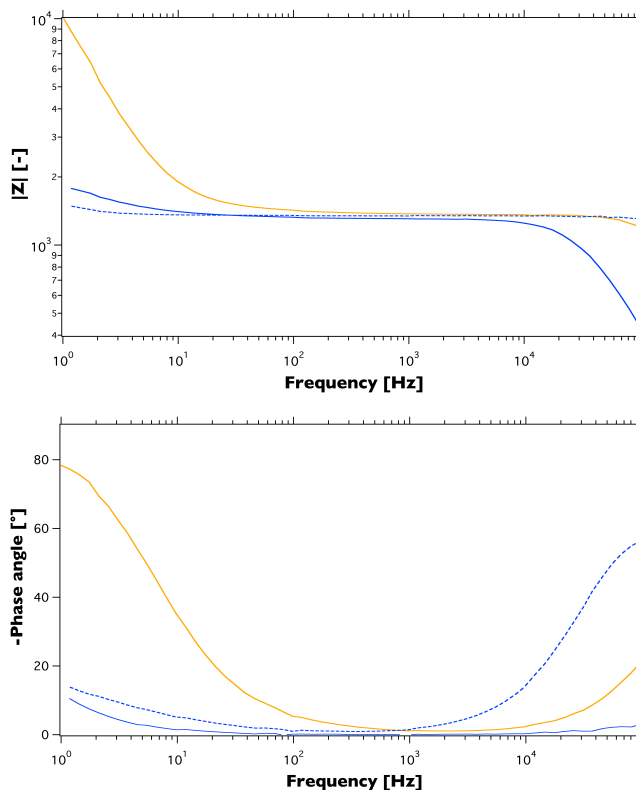


Figure 5.20: Complex Plane bode plots of module and phase angle for bare gold (—) and copolymer (— ; 50 mC/cm^2) films recorded in 0.1M NaCl using a perturbation amplitude of 5mV, measured at open circuit potential (—) and at a potential of +0.6V (---) vs Ag/AgCl.

5.4.1.4 Analysis of the controlled liberation from polypyrrole bilayers

Figure 5.22 shows the liberation of pTS^- from single polypyrrole films by cyclic voltammetry versus the number of CV cycle, and the influence of a modified copolymer film synthesized as an outer layer. The same previously described trend for the liberation of dexamethasone from polypyrrole films is observed. For modified electrodes, the liberated quantity of pTS^- anions after 50CV is smaller than in the case of single layers. It is attributed to the presence of this outer layer, which acts as impediment to the anion diffusion outside the film. Moreover, the concomitant exchange properties from both films of the bilayer structure can contribute to the decrease of released quantity. Even so, the possibility to release anions of an inner layer through a modified outer layer is shown here.

5.4.1.5 Fluorescence analysis of copolymer single and bi-layers functionalization

Analyses were performed on 50/50 copolymer films prepared with a passed charge density of 50 mC.cm^{-2} as single layers or the outer layer of a bilayer structures. After synthesis, sam-

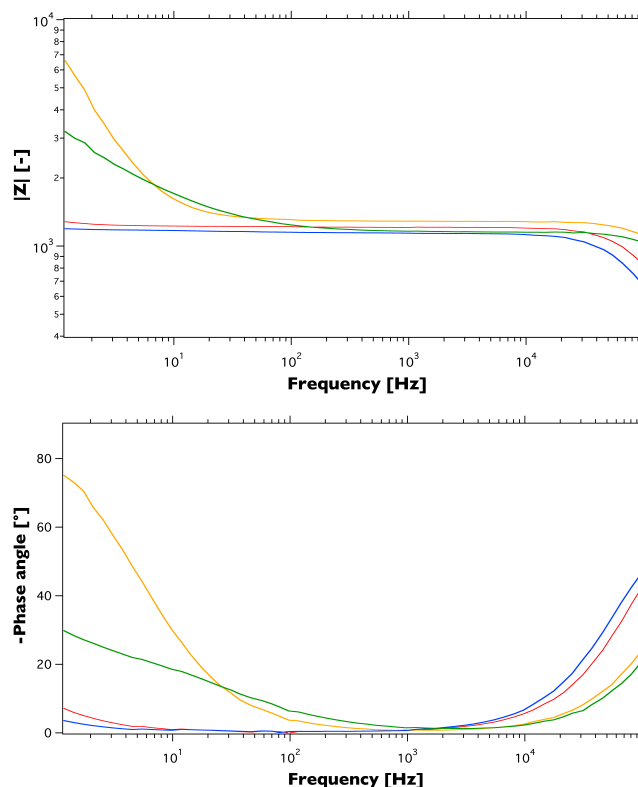


Figure 5.21: Bode plots of module and phase angle for bare gold (—), PPy/pTS (—; 100mC/cm^2), PPy/pTS + copolymer (—; 50mC/cm^2) and PEG grafted bilayer (—) films recorded in 0.1M NaCl using a perturbation amplitude of 5mV, measured at open circuit potential.

ples were reacted with amino-ended biotinylated PEG chains and further incubated with *streptavidin*^{FITC} prior to characterization by fluorescence microscopy (*cfr* material and methods). Figures 5.23 present fluorescence microscopy pictures of copolymer outer layers reacted with amino-ended biotinylated PEG chains incubated with *streptavidin*^{FITC}. The same samples are used as references with simple adsorption of PEG chains incubated with *streptavidin*^{FITC} (*ie* without the EDC-NHS step of modification). On figure 5.23 we see that the fluorescence of sample submitted to each step of chemical modification is higher than reference samples. From this, we conclude that there are carboxylic acid functions accessible for further chemical modification on copolymer films as single (developed in the previous chapter) and bi-layer structure.

Figure 5.24 shows the fluorescence intensity measured per surface unit on both copolymer and bilayer films, based on fluorescence microscopy observations. The fluorescence intensity reflects the binding of grafted biotinylated-PEG with *streptavidin*^{FITC} on the outer surface of single or multi-layer polypyrrole films. On this graph, we can see the difference in fluorescence between simple PEG adsorption (without the EDC-NHS step) and grafting (with the EDC-NHS

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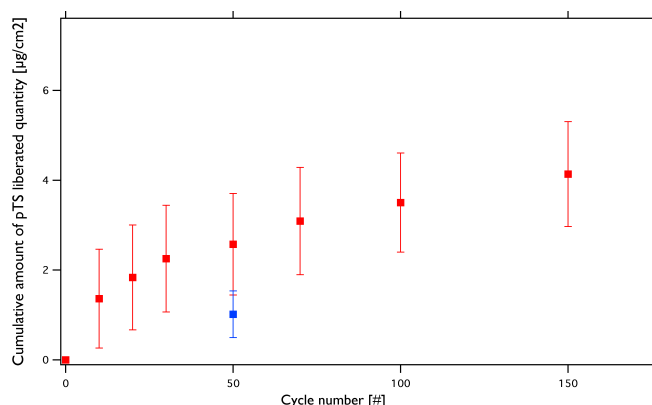
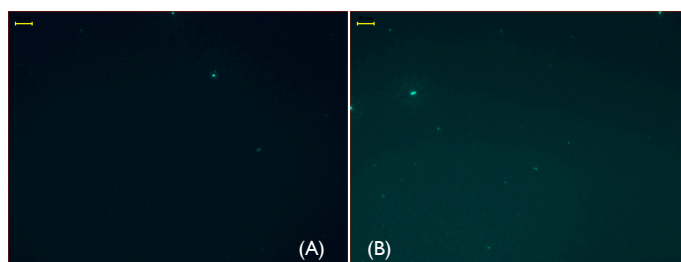


Figure 5.22: Release profile of pTS^- ions from plane PPy films ((—) PPy/pTS: $100mC/cm^2$; (—) PPy/pTS: $100mC/cm^2$ + copo: $50mC/cm^2$ modified with PEG molecules) determined by UV spectroscopy at 220nm, in function of the number of CV cycles ([-0.5V - 0.5V], 100mV/s).

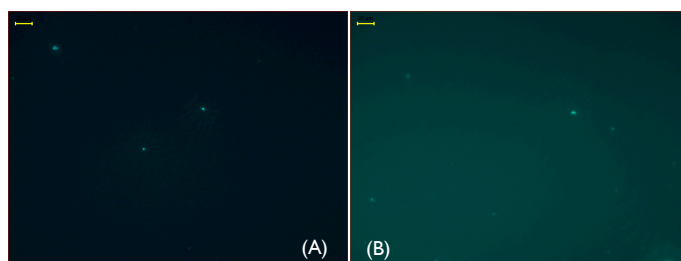
step). In addition, electrical stimulation was applied to grafted films in the form of voltammetric cycles with the same parameters as for the liberation analysis (see materials and methods). This appears to have an effect on the grafting of PEG molecules onto the surface, revealing a lower fluorescence intensity after stimulation. This can be attributed either to the release of simply adsorbed PEG molecules or to the rupture of the bonded PEG molecules on the outer copolymer film. Nevertheless, the fluorescent intensity of the remaining modified electrodes is higher than the one of non-covalently linked biomolecules.

This is an interesting property, as the aim of this kind of molecules is to combine the local drug release from the inner layer (containing molecules of interest ; by cyclic voltammetry or another electrochemical technique) and the grafting of molecules with peculiar biological characteristics onto the surface of the same electrodes on a relatively long-term basis.

5.4. Results and discussion: electrosynthesis of multi-layer copolymer films



(a) Single PPy/Py-3-COOH layer on gold (50 mC.cm^{-2} ; 0.7V versus (Ag/AgCl)).



(b) Bilayer on gold (PPy/pTS 100 mC.cm^{-2} + PPy/Py-3-COOH 50 mC.cm^{-2} ; 0.8 and 0.7V versus (Ag/AgCl))

Figure 5.23: Fluorescent optical images of different films synthesized on gold electrodes, after chemical coupling with amino-ended biotinylated PEG via EDC/NHS ((A) reference (no coupling) ; (B) EDC-NHS coupling) and incubation with *streptavidin*^{FITC} (scale bare $20 \mu\text{m}$).

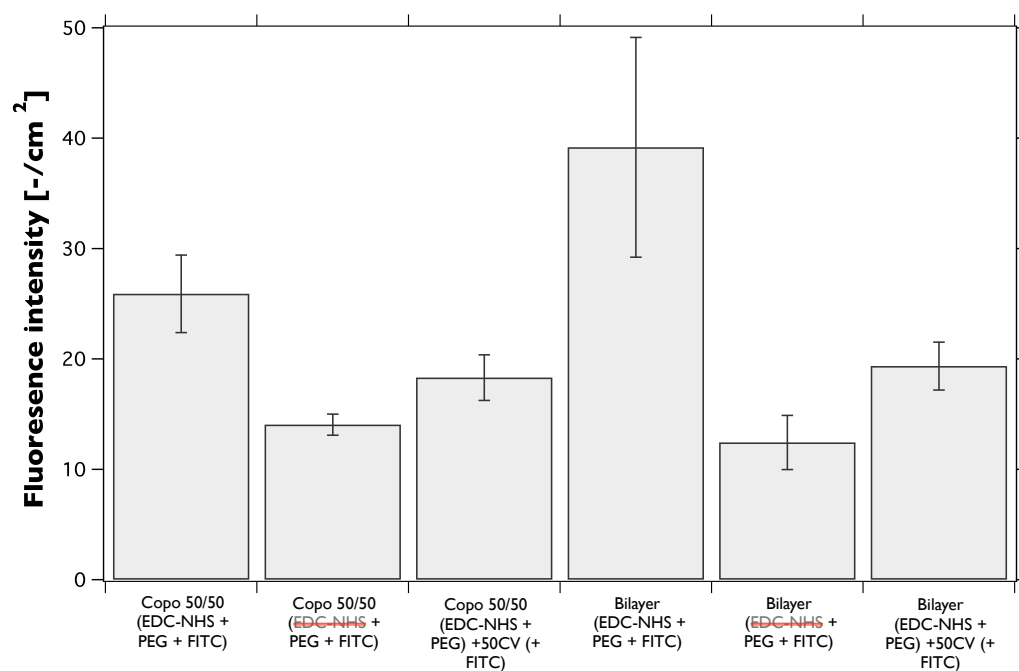


Figure 5.24: Fluorescent intensity measurements per surface unit of single and bilayer copolymer films synthesized on gold electrodes, with chemical coupling with amino-ended biotinylated PEG via EDC/NHS and incubation with *streptavidin*^{FITC}, without chemical coupling and after 50 voltammetric cycles ([+0.5 ; -0.5V] ; 100mV/s).

5.5 Conclusion

In this work, we showed that the composition of Py-co-Py-COOH copolymer films can be tailored by playing on different independent electrosynthesis parameters, such as the applied potential during electropolymerization, the composition of the comonomer solution and the charge density passed for the film formation. The successful incorporation of surface carboxylic acid functional groups in PPy materials and their further derivatization with primary amine-containing molecules was confirmed using different surface and bulk analysis techniques. From this study, it can be concluded that higher contents of Py-COOH units into copolymers are reached for thin copolymer films synthesized from Py-COOH rich comonomer solutions ($\geq 50/50$), at low applied voltage (0.7V versus Ag/AgCl), so avoiding the drawback of strongly reducing the electrical conductivity due to overoxidation. In a second time, we showed the possibility to synthesize multilayer polypyrrole structures for the simultaneous controlled delivery of molecules and grafting of biologically active groups at the surface of the outer layer. This technique allows to tailor electrode surface properties with the aim of a better integration of stimulation electrodes into a complex biological environment.

The synthesis parameters were evaluated by quartz crystal microbalance in the overview of doping level and thickness. The influences of the copolymer synthesis on both the electroactivity and the liberation properties of the inner layer were assessed by cyclic voltammetry and electrochemical impedance spectroscopy.

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6 Conclusions and perspectives

All the way down to the identification of solutions to insure a long-term integration of surgically implanted stimulation electrodes, researchers have come to the conclusion that the determining role relies on the tissue-electrode interface. Based on this, the modification of stimulation electrodes surface have been shown to successfully enhance the body response to the surgical placement of the implants, by regulating inflammatory reactions. Furthermore, three approaches have been brought forward as guidelines for the future work to be done. In the present work, those three surface modification approaches were studied in the general aim of reducing inflammatory response to the implantation of a CUFF electrode on a vagal nerve, for the therapy of Epilepsy.

In the first chapter of the present thesis, a well-defined structuration of the metallic electrodes at the nanoscale was studied. Based on a hard-templating method, nanowire arrays of platinum and gold were synthesized by an electrochemical technique. The possibility to obtain structures with pillars of controlled density, diameter and height was shown. This control was applied based on the charge passed during chronoamperometric synthesis (for platinum) or on the number of CV cycles in cyclic voltammetry. The structures were observed and characterized by SEM microscopy. Electrochemical characterization techniques were used to evaluate the electrical properties of these peculiar structures. It was shown that the impedance properties and current conductivity of nano-featured electrodes are improved, and that the size of the pillars has an influence on this trend. However, there are questions that still need to be addressed, especially concerning the diffusion-controlled electrochemical processes. In this perspective, the effects of pillar density on diffusion-controlled electrochemical systems in particular (and electrical properties in general) need to be determined. In a second time, the effects of this controlled (model) nanotopography on pre-osteoblast cells adhesion, morphology and growth was studied. It was first shown that the presence of gold nanopillars on the surface significantly affects cell adhesion, morphology and metabolic activity. On nanowire arrays, cells adopt an elongated morphology and their metabolic activity is reduced. Cell filopodia formation also showed a unique response to the created nanotopographies, and a link could be observed between the

density of nanopillars at the interface and the dimensions of cell protrusions.

This work offers many perspectives in the fundamental and application fields. First, it is believed that those kind of nano-featured electrodes could enhance the recording and stimulation properties because of their lowered impedance. Moreover, using them in an in-vivo medium, interactions between cells and topography could be tailored as an alternative signaling mechanism, in the sense of tuning cell functions (ex. cell death, attachment, genetic manipulation, adhesion, migration, polarization ...). This study thus contributes to the understanding of cell response to nanoscale cues of well-defined dimensions. It is also a promising approach for preparing biomaterials with well-defined nanotopography for controlling and guiding cell behaviours.

Furthermore, the nanofabrication technique used in this study is not only limited to the preparation of surfaces with vertically aligned gold nanopillars of well-controlled and tunable dimensions, but it can also be applied to grow nanopillars of a large variety of compositions (metals or polymers) that could also further be selectively chemically functionalized. For example, platinum will be used in the final application on cuff electrodes, as the possibility to synthesize pillars of this material with specific dimensions was demonstrated. Actually, physical nano-scale modifications could also be superimposed upon other modes of surface modifications. While nanostructuring the surface, bulk properties of the materials may not be significantly changed, or even improved depending on the applications aimed.

Going forward with the understanding of cells to vertical gold nanopillars interactions, future work can be directed to the effect of other pillar parameters (height, material, etc.). Moreover, considering the electrical conductivity of the nanostructured surface, it may be interesting to see the influence of electrical stimulation on cells behaviour. Finally, chemical modifications can be imagined, such as the grafting of recognition peptides (ex. *RGD*) on the top of every pillar.

In the second chapter, we first presented the development of a method allowing the easy and reproducible fabrication of switchable PPy-based nano-bioelectrodes for local and electrically controlled delivery of anti-inflammatory dexamethasone molecules. The drug incorporation was done using a one-step electropolymerization of a PPy film onto an electrode covered by a brush of metallic nanopillars. The release of the drug was carried out using cyclic voltammetry stimulus and was found to be electrically controllable. The presence of the Pt nanopillars onto the electrode substrates improved the mechanical stability and the adherence of the polymer onto the metallic substrate. Both of these parameters are of great importance for the development of long-term performances neural stimulation electrodes. The release was found to follow a first order quantitative release in function of the number of applied CV cycles, as well as a dependence on other technique-related parameters (scan rate, potential window). However, the limits of the in-vivo use of cyclic voltammetry were enlightened and at the same time reproducibility problems for the properties of the films.

We described the optimization of the fabrication technique to overcome these problems, and the liberation properties of the two-layers polypyrrole films. Biphasic potential pulses stimulation

was studied for the liberation of dexamethasone in-vivo, with the restrictions linked to the complexity of the biological environment. A device for the application of biphasic potential pulses was developed simultaneously to the study of the parameters influencing the liberation. The active liberations of dexamethasone either by continuous stimulation or by small series of pulses were confronted, with the interesting outcome that short series are more effective and safe for the properties of the films. The evolution of passive liberation in time following an active phase of stimulation was then described. Finally, a day-by-day release profile was shown, and the interesting parameters enlightened for future developments of the release device. Based on this, a first model of in-vivo experiments were conducted, with encouraging results about the reduction of inflammatory reaction on CUFF implanted rat sciatic nerves.

However, further improvements of electro-chemical stability and drug releasing properties are expected, especially in the reproducibility of the films properties. The relation between the electrical charge and the quantity liberated has to be further investigated. Likewise, the control over the potential applied in-vivo has to be perfected, and furthermore the diagnostic of the state of the electrode at a certain time, to ensure its long-term performances.

Though, in this work, we studied the delivery of DEX, the nano-bioelectrodes can be easily adapted for the delivery of a wide range of biomolecules and drugs, and are therefore expected to be of interest for a variety of electrode/tissue interfaces in biomedical devices. More extensively, the development of new devices for the in-vivo stimulation using polypyrrole-coated metallic electrodes by biphasic potential pulses with determined parameters has a lot of concrete medical applications in perspective.

In the last chapter of this thesis, we presented a fabrication process of copolymeric films with specific chemical groups allowing further modifications. We demonstrated that the composition of Py-co-Py-COOH copolymer films can be tailored by playing on different independent electrosynthesis parameters, such as the applied potential during electropolymerization, the composition of the comonomer solution and the charge density passed for the film formation. The further successful derivatization with primary amine-containing molecules was confirmed using different surface and bulk analysis techniques. In a second time, we showed the possibility to synthesize multilayer polypyrrole structures for the simultaneous controlled delivery of molecules and grafting of biologically active groups at the surface of the outer layer. This technique allows to tailor electrode surface properties with the aim of a better integration of stimulation electrodes into a complex biological environment.

It was also shown that this synthesis and the further modification of the outer surface is compatible on one side with the controlled release of the inner molecules and on the other side that this release does not affect irreversibly the surface modification. Even if this has to be studied in more details, it is a first step on the use of electrodes with a combination of several simultaneous surface modifications.

The fabrication of an electroactive thin polypyrrole film on which can be grafted biologically

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active molecules in a defined density is the promise of many applications in the field of electroactive implants. The modification technique is well-known and simple, and allows the covalent grafting of many molecules, with specific properties: cell growth factors, proteins, antibodies, etc.

During this work, we first explored three approaches of surface modifications individually. In a second time, some combinations of those modification approaches were presented. At this point we saw that even though we have to be careful in the use of them (and in their respective limitations), they could be used at the same time as a complex electroactive nanostructured electrode for the controlled delivery of (bio-)molecules and with a tailored surface. In this aim, it is necessary to have an extended control over the electrochemical properties of the synthesized films. The parallel development of the device for the electrical stimulation and drug release is of the greatest importance to pave the way to the new generation of electroactive implantable autonomous devices.