

semi-crystalline polymers or thicker films may behave differently. Therefore, further study is needed to fully optimize the lift-off process and guarantee the structural integrity of the films.

All-polymer dual-patterned surfaces can be designed to have spatially-resolved stimuli responsiveness. The patterned pHEMA and pPFA surface is ideal for demonstrating this behavior. Figure 3.7. shows an AFM image of microwells having a depth of 385 nm (line scan shown). Where, the material inside the wells is pPFA and the material separating the wells is pHEMA. Since the pHEMA swells when immersed in water while the pPFA does not, their different swelling capabilities can be used to actuate the depth of the microwell. AFM imaging using a wet cell revealed that the differential swelling resulted in a ~30% increase in well depth, reaching a step height between both materials of approximately 500 nm, as shown in Figure 3.8.

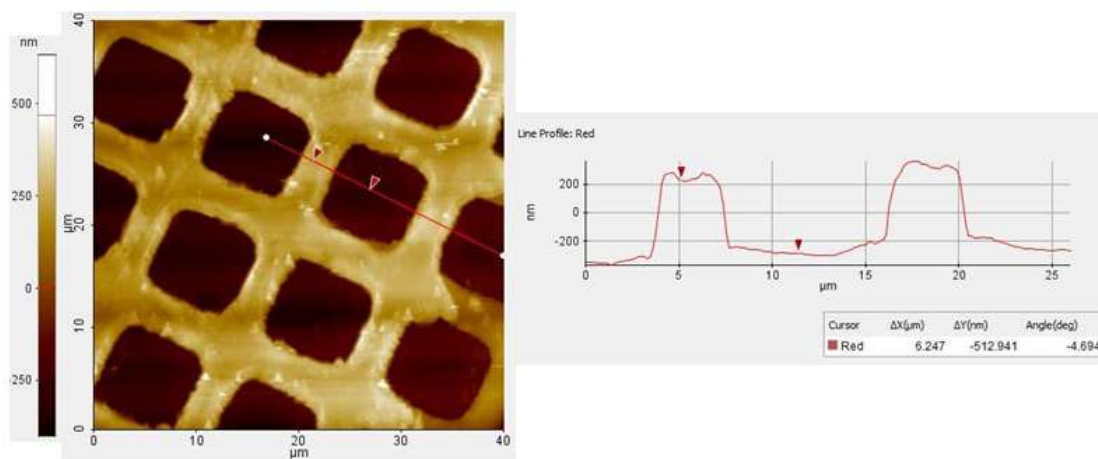


Figure 3.8. AFM of the microwells in its swollen state.

A line scan between the points marked with arrows reveals a step height of 513 nm.

AFM in Figure 3.9 show a typical 3D image for the patterned surface resulting from this technique. The image again showed the capability of pHEMA film to swell even the presence of hydrophobic depressions of PFA.

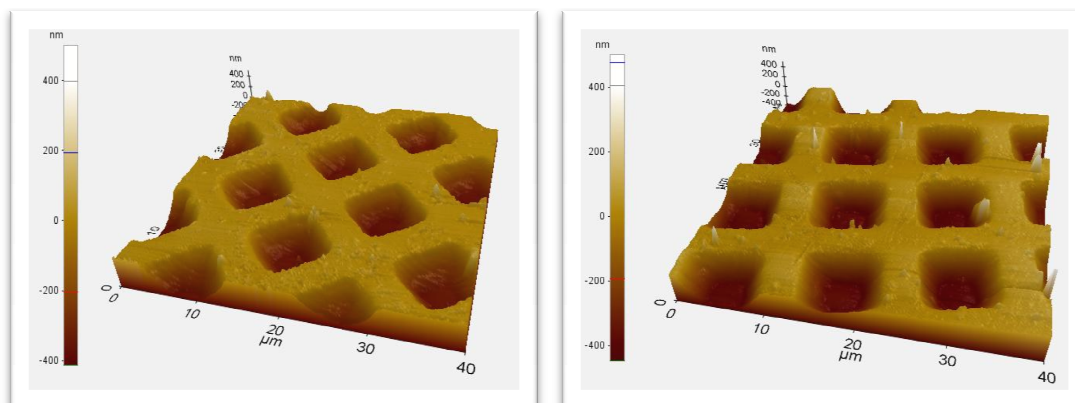


Figure 3.9. 3D AFM image of hydrophobic depressions in a hydrophilic matrix in its dry state (left) and swollen state (right).

3.4. Discussion

The patterning process to obtain bifunctional surfaces presented in this chapter involves a simple 4-step process that includes the deposition of a first polymer, masking and etching step, deposition of a second polymer and finally lift-off. This simple technique, which is independent of the chemical nature of the polymers, allows engineering the topography by controlling the relative thickness of the two polymer films. The dual-use mask, acting as both etch mask and a lift-off mask, resulted in an inherently self-aligned pattern in a single lithographic step. This method has the advantage of allowing microfabrication of patterned surfaces in a fast and specific manner, when compared to other available methods. Micro-fabrication techniques have found a wide range of applications in the fields of electronics, optics and biotechnology. The technique presented here not only allows the fabrication of topographic reliefs in a fast and efficient way, but it also enables the design of 3D features having defined spatial chemical patterns. Careful choice of suitable monomers should allow the design of surfaces having special features, such as wetting behavior, anti-biofouling activity, cell attachment, among others. Topographic micro-features are commonly found in the nature and may serve as inspiration for the design of materials with superior properties. For example, surfaces that control wetting via microcondensation occur naturally in nature (Garrod et al. 2006; Parker & Lawrence 2001), the *Stenocara* beetle's back is used to collect water in the desert and have inspired the fabrication of hydrophilic-hydrophobic patterns for applications in microfluidics and harvesting. Commonly, biomimetics is the field that deals with development of products that are inspired by nature.

Once it has been demonstrated the viability of the technique to deposit pHEMA hydrogels and its simplicity to create both structured and randomized surfaces together with the possibility of a further modification to obtain the desired end-properties, the next logical step is implementation of these surfaces in a real sensing device.

Chapter 4.

Hydrogel Thin Film Coatings For Stable Sensor-Tissue Interface

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4.1. Introduction

Chapter II and III have proved the ability of the iCVD technique to deposit thin polymer films on a wide range of substrates with excellent deposition control and independently of the geometric features of the surface. This advantages open up the possibilities of using these thin hydrogel films as protective layers for biosensors in *in vivo* applications. This chapter aims to validate the potential of these techniques in a real *in vivo* application by preventing the degradation in sensitivity and response time.

The development of minimally invasive and short-term implantable microelectrodes for on-line tissue monitoring is a field of increasing clinical interest. In different disciplines, such as retinal signaling or neuroscience research, the quality of their recordings depends critically on microelectrode impedances. In particular, high-impedance electrodes are associated with increased thermal noise and signal loss (Loeb et al. 1995; Ludwig et al. 2006; Shoval et al. 2009). Additionally, high electrode impedances limit the charge injection for stimulation experiments (Keefer et al. 2008).

Significant efforts are being invested in the modification of the microelectrode materials or their surfaces in order to improve the contact of the electrode-electrolyte interface in biosensors. To the best of our knowledge, despite numerous-great efforts, an interface that maintains a stable recording tissue interface in long term experiments has not been achieved yet. As previously reported (Heim et al. 2011), it is possible to enhance the sensitivity of the microelectrodes by increasing their surface roughness via deposition of black platinum, poly-Si, titanium nitride and carbon nanotubes, among others. However, these new deposited layers are poorly adhered to the electrode surface and might be lost during operation, leading to unacceptable values of impedance. This limitation might be overcome by depositing thin films

of polymeric hydrogels that provide sufficient protection and adhesion to the electrode coating material. The ultrathin film deposition of the hydrogel poly(hydroxyethyl methacrylate) (pHEMA) by initiated chemical vapor deposition (iCVD) described in Chapter 2 is presented in this study as a technological breakthrough to improve the mechanical properties of the microelectrodes.

There have been many reports in the literature of different microelectrode surface modifications investigated to improve the quality of the biosensors measurements. These methodologies are able to improve the microelectrodes' performance without changing the electrode's projected area due to the increase of the surface roughness.

On the other hand, the studies of passive electrical properties of biological tissues based on electrical bioimpedance measurements have shown that the electrode impedance can greatly influence the quality of the experimental results (Grimnes & Grottem 2008). For bioapplications, devices that require the use of micrometric electrodes are typically demanded (Suselbeck et al. 2005; S. B. Cho & Thielecke 2005). Nevertheless, the use of microelectrodes usually entails high impedances, which are out of the range for the desired applications, since impedance increases with decreasing electrode area. The impedance measurements can be performed using a two-electrode method, and high impedance electrode values can give wrong information about the tissue analyzed. An alternative measuring system design is the four-electrode method (Schwan & Ferris 1968). Ideally, this method limits the parasitic effects of the electrode-electrolyte interface impedances (Grimnes & Martinsen 2007). However, this technique has an important limitation because the impedance of the current electrodes produces a common mode voltage that the differential amplifier must reject. Therefore, the electrode-electrolyte impedance values shall be at least in the same order or preferentially 10-

fold higher than the tissue measured impedance. The most common approach to overcome this limitation is by increasing the surface roughness of electrodes (Ivorra et al. 2003).

Electrochemical deposition of black platinum (Robinson 1968; Marrese 1987) and gold (Priano et al. 2008; Huang et al. 2009) are common approaches for the modification of surface microelectrodes in order to reduce their interface impedance and improve the quality of the measurements. Other reported techniques include the roughened poly-Si deposition process (Paik et al. 2003), conductive polymer coatings (Ludwig et al. 2006), titanium nitride (Janders et al. 1996), iridium oxide (Cogan 2008), and alloy formation with aluminum (Davidson 2004). Furthermore, several innovative methods in the nanotechnology field aimed to decrease electrode impedance have been developed, such as the use of carbon nanotubes coatings (Gooding 2005). Each one of the mentioned techniques allows the site specific electrode modification and have certain advantages and limitations. Significant efforts are still underway in order to achieve a universal interface with optimal selectivity, sensitivity, good charge transfer characteristics and long-term recording (Negi et al. 2010; Ferguson et al. 2009).

The feasibility of electrochemical deposition of black platinum onto surface electrodes has been previously demonstrated (Ivorra et al. 2003). Recently, a simple deposition method for modifying platinum (Pt) electrodes with carbon nanotubes SWNTs (Gabriel et al. 2008; Gabriel et al. 2009) and grown carbon nanotubes (Martin-Fernandez et al. 2009) has been reported. The use of different sensor configurations usually highlights the relative fragility of the black platinum and the carbon nanotube layers. This leads to electrode degradation, limiting the reuse of the device until the electrode impedance increases out of the range of the application. In addition, detachment of coating material to the tissue may be detrimental in *in vivo* applications.

Thus, in order to avoid all the mentioned problems a new coating strategy is necessary for the preparation of stable and robust sensors. The present work is focused on demonstrating the feasibility of the ultrathin films deposition of the hydrogel pHEMA by initiated chemical vapor deposition (iCVD), the process proposed is schematically shown in Figure 4.1. As it has been mentioned before, pHEMA is a well-known biocompatible material (Chirila et al. 1993; Lloyd et al. 2001). The physical properties of pHEMA hydrogels make them attractive for a variety of biomedical and pharmaceutical applications, such as the coating of sensor devices. pHEMA biocompatibility allows to be considered for applications whereas the hydrophilicity can impart desirable release characteristic. Moreover, surfaces modified with pHEMA have demonstrated low protein adsorption, due to decreased non-specific interactions (Martins et al. 2003; Bayramoglu & Arica 2005) making this polymer an attractive material to be in contact with biological fluids.

We aim to demonstrate the possibility of depositing pHEMA hydrogels over a previously black platinum modified electrode to improve its mechanical properties. With this hydrogel is easy to obtain strongly adherent coatings for the preparation of short and long-term stable and biocompatible sensors. The main objective is to use pHEMA hydrogels to obtain strongly adherent coatings for the preparation of short and long-term stable and biocompatible sensors.

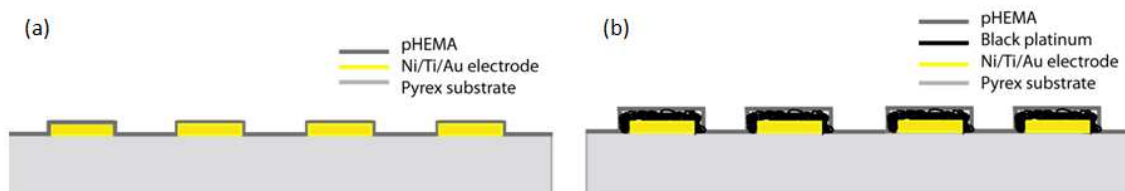


Figure 4.1. Schematic profile of the electrode area and coating of the hydrogel.

(a) without and (b) with black platinum.

4.2. Experimental Section

4.2.1. Fabrication of an Impedance Sensor and its Modification with pHEMA Hydrogel

Sensor Fabrication

The impedance sensor was fabricated using conventional microsystems technology (Heer et al. 2004). The sensor was entirely fabricated in the clean room facilities at the National Center of Microelectronics in Barcelona (Spain) through standard photolithographic techniques using 500- μm -thick Pyrex 7740 wafers as the substrate material. A photoresist was spin-coated and UV-exposed on a previously deposited Ni/Ti/Au layer (50/50/150 nm) to form a pattern (via wet-etching methods) the electrodes and their connection tracks. Next, a passivation layer was deposited onto the wafer. For this layer, a $\text{SiO}_2/\text{Si}_3\text{N}_4$ (300/700 nm) layer was coated with plasma-enhanced chemical vapor deposition (CVD), and a second photolithographic step was used to etch the deposited layer into a pattern on the electrode area and connection pads. Finally, the wafer was sawed, the connection wires were bonded directly onto the device and the connections were covered with a biocompatible epoxy resin (Epo-Tek OG147-7) to waterproof the zone.

Two different impedance sensors were used. Figure 4.2. (a & b) shows a planar-sensor that consists of eight gold electrodes arranged parallel between them with different separations as can be seen in Figure 4.2. The electrode surface area was 30 μm width, 2000 μm length, 0.25 μm height and had an area of 0.06 mm^2 . Figure 4.2. (c & d) displays a micro-needle that measured 14.85 x 0.83 mm^2 , and each device had two pairs of 300 x 300 μm^2 Pt electrodes separated by 280 μm .

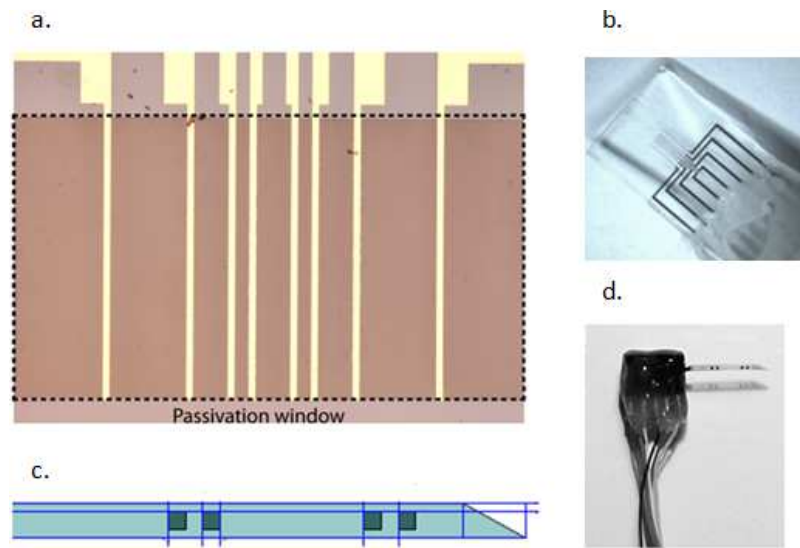


Figure 4.2. Images of both impedance sensors.

- (a) Optical images of the electrode area. (b) Planar-sensor impedance picture. (c). Schematic profile of the micro-needle area. (d) Micro-needle impedance picture.

Electrode Modification with Black Platinum

Gold electrodes on individual devices were electrochemically coated with a porous layer of black platinum to reduce their impedance through a customized process of platinization. Platinization was carried out using a Pt electrode (Radiometer Analytical) in a LC20H ultrasonic cleaner (Elma) and involved an initial cleaning step of the electrode surface for 3 min in ethanol. Thereafter, electroplating was performed for 3 min in a solution containing platinum chloride (hydrochloric acid 0.1 M, 2.3% platinum (IV) chloride and 0.023% lead (IV) acetate 99%) at 200 μ A on all electrodes simultaneously. All reagents used in this process were analytical grade (Panreac) and used as received.

Electrode Modification with pHEMA Deposition

Films were deposited on sensors in a custom built vacuum reactor. As mentioned before, in the iCVD process, thermal excitation was provided by resistively heating a Nichrome filament

(80% Ni - 20% Cr) mounted in a parallel array and which provided thermal energy for selective decomposition of molecules.

The filament holder straddled a cooled stage on which the sensor was placed and was sufficiently large to ensure uniform deposition over the area. All samples were maintained at 30°C through backside coolant circulation. The distance between the filament and the stage was set at 2.2 cm. Pressure in the reactor was maintained at 0.5 Torr.

The HEMA monomer (Sigma Aldrich, 99+%) and the cross-linking agent ethylene glycol diacrylate (EGDA, Sigma Aldrich, 90%) and the initiator were used without further purification. HEMA and EGDA liquids were vaporized in a glass jar, which was heated to 80°C and 70°C respectively. The EGDA and HEMA vapors were fed into the reactor through a line maintained at 90°C. TBPO (Sigma Aldrich, 98%) was vaporized in a glass container at room temperature and fed through a different port. All vapors were mixed together before entering into the reactor. The flow rates of HEMA, EGDA and TBPO were regulated using needle valves, and were kept constant at 2 sccm for the monomer (HEMA), 1 sccm for the cross-linker and 1 sccm for the initiator (TBPO). Films were deposited at a filament temperature of 300°C. The temperature was measured using a thermocouple directly attached to one of the filaments.

In order to analyze the influence of the film on the electrodes two different thicknesses of pHEMA hydrogel, 100 and 200 nm, were electrically tested on the sensors without black platinum. The study was done with both impedance sensors; micro-needles (experimental series N) and planar-sensors (experimental series S). The difference between coatings of 100 nm and 200 nm were compared to choose the best option. The resulting thickness was used as an experimental condition for the rest of depositions.

Table 4.1. Experimental conditions for planar-sensors (series S) and micro-needles (series N).

Letter H represent the samples with pHEMA films.

Sample Name	Thickness (nm)
S1 & N1	-
S2 & N2	-
SH1 & NH1	100
SH2 & NH2	200

4.2.2. Electrode-Electrolyte Impedance Characterization

Electrochemical impedance spectroscopy (EIS) was carried out in order to characterize the electrode-electrolyte interface impedance of the electrodes. EIS was performed with a custom impedance analysis system (Guimera et al. 2008) by applying a maximum of 70 mV AC and 0 DC. The electrode impedances with and without black platinum and pHEMA were estimated with the common two-electrode method by measuring the impedance and phase shift versus frequency from 100 Hz to 100 kHz in physiological saline solution (0.9 wt. % NaCl, with a nominal resistivity of $71.3 \Omega \cdot \text{cm}$ at 25 °C) against a Platinum reference electrode (Radiometer Analytical). As showed earlier in 2.3.3, the synthesized films exhibited a rapid, reversible swelling response, while maintaining adhesion to the substrate. As characterized by *in situ* spectroscopic ellipsometry, the films reached their equilibrium of swollen water content within 5 min of incubation in pH 7.4 buffer solution (Baxamusa et al. 2008). All impedance measurements were carried out once the equilibrium was reached.

Data were expressed as average values \pm standard deviation and significant results were determined by a 95 % confidence interval.

4.2.3. Mechanical Stability Test of Electrodes

Two simple mechanical tests, consisting of either manual repeated insertions and extractions of the device in a polyurethane foam layer (Chemtronics ref. 41050) or a vibration sonication in an ultrasonic bath during 15 seconds (Branson Ultrasonics, 35 kHz), were carried out to induce detachment of the fragile black-platinum. These procedures have been considered aggressive enough in comparison with the implantation process of the electrodes in a living tissue, in order to test the mechanical stability of the different coatings over the electrode.

4.2.4. Electrode Surface Analysis

To characterize the black platinum and pHEMA coating, the initial electrode surface with and without the hydrogel thin film was inspected with a scanning electron microscope (SEM, Hitachi S-530). In order to obtain the profile at the electrode area of the different layers deposited over the electrode the focused ion beam electron microscopy (FIB/SEM, Carl Zeiss Crossbeam 1560XB) techniques were employed for their characterization. All images shown were tilt corrected.

4.2.5. Evaluation of Impedance Sensor Performance *in vivo*

The study was performed under the supervision of the University of Valladolid ethics committee, and it conformed to the EU guidelines for handling and care of laboratory animals. For these experiments, a total of 10 New Zealand white rabbits that weighed between 2.5 and 3 kg were used. The rabbits were anaesthetized with an intramuscular injection of 50 mg/kg of ketamine (Imalgene 1000[®], Merial) and 7 mg/kg of Xilacine (Rompun[®], Bayer). Next, the

animals were housed individually in cages, and their heads were stabilized to facilitate lateral access to the eyes, which simplified the experimental process and sensor application.

The mechanical stability of the post-processed electrodes, with and without pHEMA, was also tested in *in vivo* experiments. In order to perform the measurements at a stable pressure of 40 mm Hg, the impedance planar-sensor was attached to an applanation tonometer tip (AT 900®; Haag-Streit). The proposed sensor came into contact with the cornea surface of a rabbit eye for 2 minutes, in order to assess the corneal permittivity. In consideration with the topic presented herein the contact was repeated up to ten times per eye, and the measurements were done in ten rabbits.

4.3. Results

4.3.1. Characterization of Electrode Surfaces by SEM Analysis

The passivation layer window has been specially designed for the study of the pHEMA coating and is not individualized for each electrode. In this manner, the electrode is not planar because the top surface and both sides of the electrodes are exposed. This configuration increases the mechanical instability of the electrodeposited black platinum layer. A simple inspection of the electrode area is enough to demonstrate the presence (or absence) of the black platinum over the electrodes due to their black appearance (Figure 4.3.). The optical inspection after pHEMA deposition does not denote any difference.

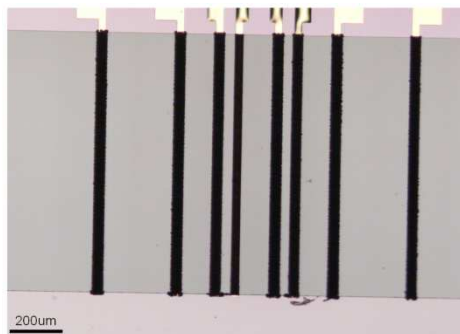


Figure 4.3. Black platinum deposited on gold electrode surface.

Image denotes the black appearance due to black platinum.

The black platinum layer, as any electroplated metal, is rough and porous as can be observed in the scanning electron SEM image in Figure 4.4. (a). As reported in literature the roughness (rms) value of this layer is in the order of 250 ± 120 nm (Gabriel et al. 2007). The fragility of this layer is explained by its high roughness, when compared with the roughness value of the bare gold electrode 7 ± 3 nm. Full characterization was performed on the pHEMA coating. The

pHEMA film was deposited over the entire device, as shown schematically in Figure 4.1. Figure 4.4. shows the SEM image of the hydrogel coating the sensor out of the electrode area indicating that this new layer is approximately 90-100 nm thick.

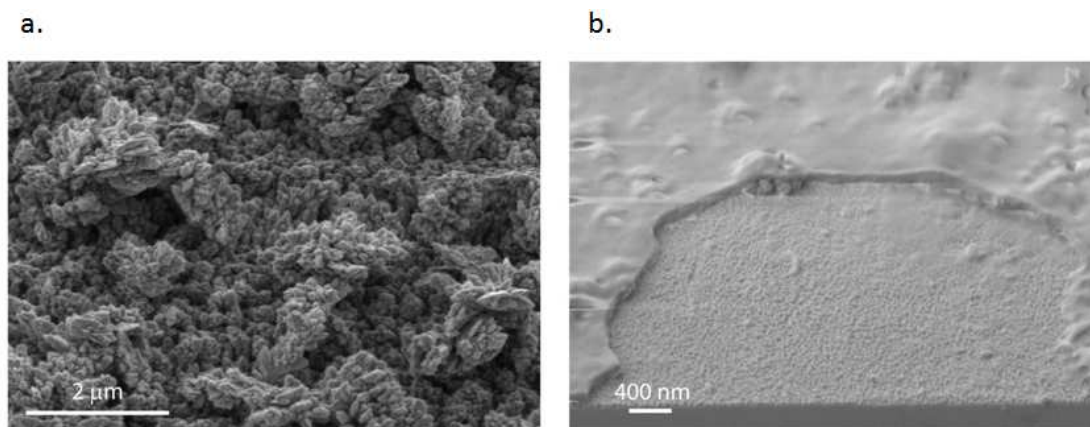


Figure 4.4. SEM images of (a) bare electrodeposited black platinum and (b) pHEMA film.

Figure 4.5. compares SEM cross-section images of the electrode area (Ni/Ti/Au, 150 nm, over pyrex substrate) in electrodes with and without black platinum after pHEMA coating. In Figure 4.5. (a) the pHEMA ultrathin layer (90-100 nm) is clearly observed. The coating appears as a continuous film and presents a slight rough surface. In Figure 4.5. (b), the rough black platinum layer (1.5 μm) can be distinguished above the Ni/Ti/Au layer. The pHEMA layer cannot be observed due to its nanoscale thickness; however its presence can be confirmed by the soft appearance of the black platinum and also by the surface charge during SEM analysis due to the low conductivity of pHEMA layer (data not shown). The RMS values obtained for the black platinum with a pHEMA coating were in the same range as for the untreated black platinum, 239 ± 110 nm vs. 250 ± 120 nm, respectively. These results indicate that the coating did not alter the microstructures obtained with the black platinum and the high surface area was maintained. This experiment confirms that the pHEMA iCVD depositing process is not limited to planar surfaces and the hydrogel layer conformally coats the microgeometries of the black

platinum layer, which is consistent with previous experiments and modeling of the conformality of iCVD polymers (Ozaydin Ince & Gleason 2010; Baxamusa & Gleason 2008a). Thus, iCVD deposition seems to fulfill the requirement of ensuring that the advantageous high surface area of platinum black.

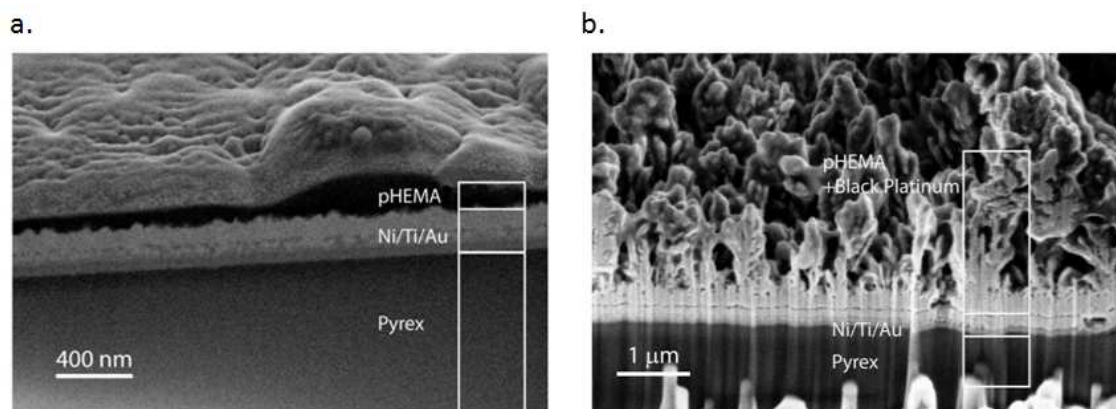


Figure 4.5. SEM images taken with FIB in the electrode area to obtain cross-section profiles.

(a) Ni/Ti/Au/pHEMA over the pyrex substrate and (b) Ni/Ti/Au/Black platinum/pHEMA.

4.3.2. Electrical Characterization of Sensors

Two important points are necessary to validate the use of the pHEMA hydrogel as coating in sensors. In one hand, ensure that the iCVD process does not damage the electrical device. On the other hand, the hydrogel thin film must be stable and allow the passage of the analytes and, as suggested before, must be conformal to the black platinum coating in order to maintain the high surface roughness advantage. To demonstrate the feasibility of pHEMA coating, the sensors response was electrically characterized in the presence or the absence of a hydrogel layer.

In Figure 4.6., it is shown the two-electrode impedance measurements at different stages of the electrode modification. As expected, the increase of the electrode surface roughness due to the platinization process and, therefore, its effective surface area, had the direct consequence of diminishing the electrode impedance module (70 fold at 100 Hz) and increasing the phase shift stabilization value ($\Delta\text{Arg}(Z) = -14^\circ$ at 100 Hz). The impedance measurement of plated electrodes after 15 days showed a slow increase (4 fold at 100 Hz) in the impedance that can be explained by the dehydration and hydration processes that this layer may suffer. The hydration level of the black platinum may affect its porous morphology, and thus, induce changes in its impedance. Impedance measurements were performed immediately after the pHEMA deposition and followed for a period of 45 days. It can be observed that for a reasonable period of time the impedance is not altered, this result encourages the viability of pHEMA as a sensor coating.

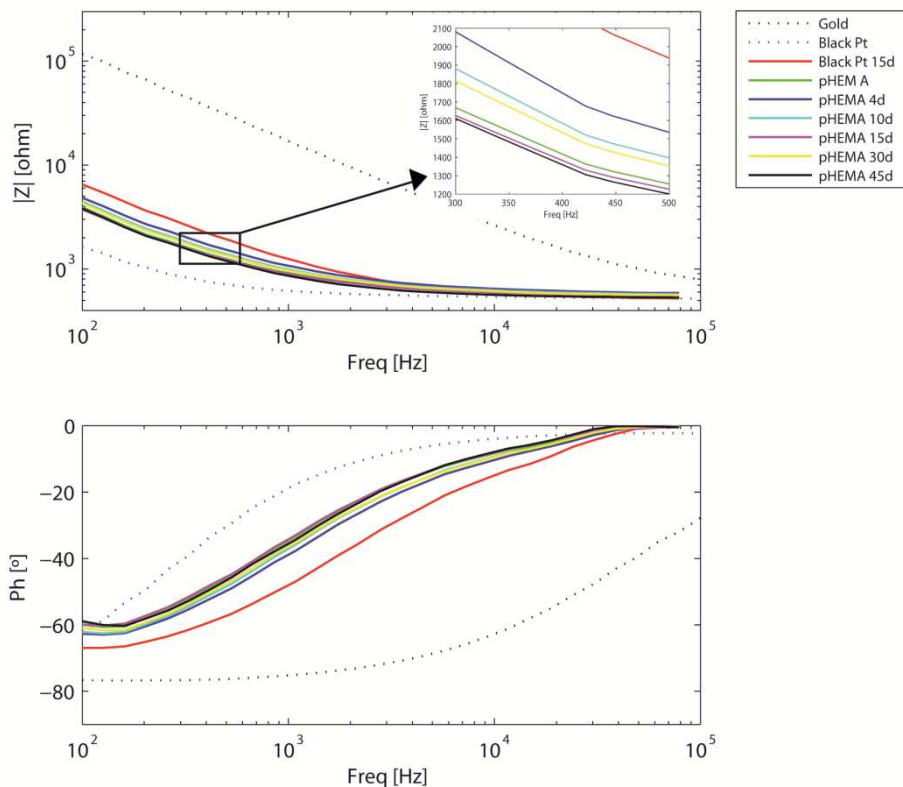


Figure 4.6. Evolution of the impedance modulus and phase as a function of frequency.

Bare gold electrode (black dotted line), electrodeposited with black platinum (blue dotted line), black platinum 15 days after the electrodeposition (red), modified with pHEMA at $t=0$ (green), $t=4$ days (blue), $t=10$ days (light blue), $t=15$ days (violet), $t=30$ days (yellow), $t=45$ days after pHEMA deposition (black).

Each curve represents the median of the 8 electrodes of one device.

Related to the phase shift stabilization value, it can be observed values higher than -90° were observed after the black platinum deposition, which indicates an increase of the electrode roughness. To understand this phenomenon from an electrical point of view, the electric behavior of the electrode-electrolyte impedance in aqueous solutions can be represented by an equivalent circuit shown in the scheme represented in Figure 4.7.

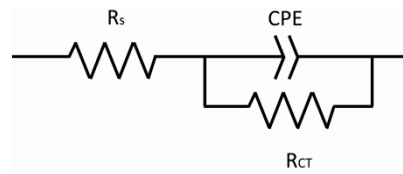


Figure 4.7. Equivalent circuit model of electrode-electrolyte interface impedance.

The electrode-electrolyte interface impedance behavior can be approximated modeled as the parallel combination of a charge transfer resistance (R_{CT}) and a constant phase element (CPE – non-faradaic pseudocapacitance). The CPE component is the best approach for modeling the double layer behavior. R_{CT} and CPE are also connected in series with the spreading resistance (R_s-solution resistance), which models the spreading current from the conductive solution into the electrode. However, for the frequency range used in this work, R_{CT} can be neglected. Impedance module could be expressed with:

$$Z(\omega) = R_s + Z_{CPE} = R_s + \frac{1}{(C_{dl} \omega j)^\alpha}$$

Equation 4.1.

Roughness changes are related with the alpha parameter (α) of the CPE component as described before (H. K. Song et al. 2000; Gabriel et al. 2008), and has a value comprised $1 \geq \alpha \geq 0$. When the constant α is equal to 1, the CPE describes an ideal capacitor ($\text{Arg}(Z) = -90^\circ$) and when the α is equal to 0, the CPE describes an ideal resistor ($\text{Arg}(Z) = 0^\circ$). The proposed fitting (data not shown) matched the measured data, validating the equivalent circuit. The α values obtained after the fitting (using Zview 3.1) were 0.80 for Black platinum 15d and coated with pHEMA 0.79, which confirms that the black platinum and black platinum-pHEMA based electrodes had higher roughness and porosity than the bare electrodes with $\alpha = 0.84$. In the Bode representation, changes in α can be observed as an increase of the phase shift

stabilization value of $\text{Arg}(Z)$ value from -76° for the based gold electrodes to -67° for Black platinum 15d and coated with pHEMA -59° .

However, it was observed in Figure 4.6. that pHEMA slightly decreases the impedance module. This phenomenon could be explained by a decrease in the thickness of double layer capacitance in the presence of pHEMA.

The impedance spectrum can be explained by the different elements in the equivalent circuit. The double layer region is in the low frequency range up to 1 kHz, and thus, the double layer capacitance dominates the impedance measurement here. From the fittings done, the values of the double layer capacitance for the black platinum without and with pHEMA are $9.2e^{-7}$ F and $1.8e^{-6}$ F, respectively. As accepted elsewhere the double layer capacitance can be expressed with the following formula (Bard & Faulkner 2001):

$$C_{dl} = \epsilon_r \epsilon_o (A/d)$$

Equation 4.2.

Where ϵ_r is the relative permittivity of the layer separating the ionic charges and the electrode; ϵ_o is the vacuum permittivity, A is the electrode area; and d is the thickness of the double layer. Considering that the electrode area and the dielectric permittivity of the medium are constant, the changes in decreasing the impedance can be explained by a decrease in the thickness of the double layer. Approximately the new distance with pHEMA implies a reasonable decrease to half the normal value considered of 3 \AA (Bard & Faulkner 2001). In addition, it is also possible to have a second effect of the pHEMA deposition on the black platinum. Even though the electrode area is constant, experimentally it has been observed that the high roughness of the black platinum (high porosity) can difficult the complete hydration of this layer. Hence, the

conformal recovery of the pHEMA hydrogel, which easily swells, could favor the hydration process increasing at the same time the contact area, which would slightly decrease the impedance value.

In order to confirm the hydration level of the black platinum, electrodes were coated with different pHEMA hydrogel thicknesses and their resulting impedances were determined. Figure 4.8. compares the impedance of coated sensors having different hydrogel thickness, 100 vs. 200 nm. The measures of impedance before and after pHEMA deposition are shown in both cases. After the film deposition, pHEMA allowed the passage of the analyte and it can be measured by the micro-needle and planar-sensor. The modification was good enough to achieve a better result with the coating as it has been mentioned before. The thickness experiment confirmed that pHEMA deposition decrease the thickness of double layer capacitance and helps in the hydration process. This phenomenon was more emphasized in the case of 100 nm than in the 200 nm. These results can be explained because the response of the device is governed by the diffusion of the analyte through the hydrogel matrix. The nanoscale thickness of the overlaying pHEMA affects to the diffusion of the analyte. Besides, it is known that the swollen water content is limited by the hydrogel thickness; this has influence on the hydration process that can be faster and more efficient in thinnest films. This is the reason why the rest of the experiments were done with a thickness of 100 nm.

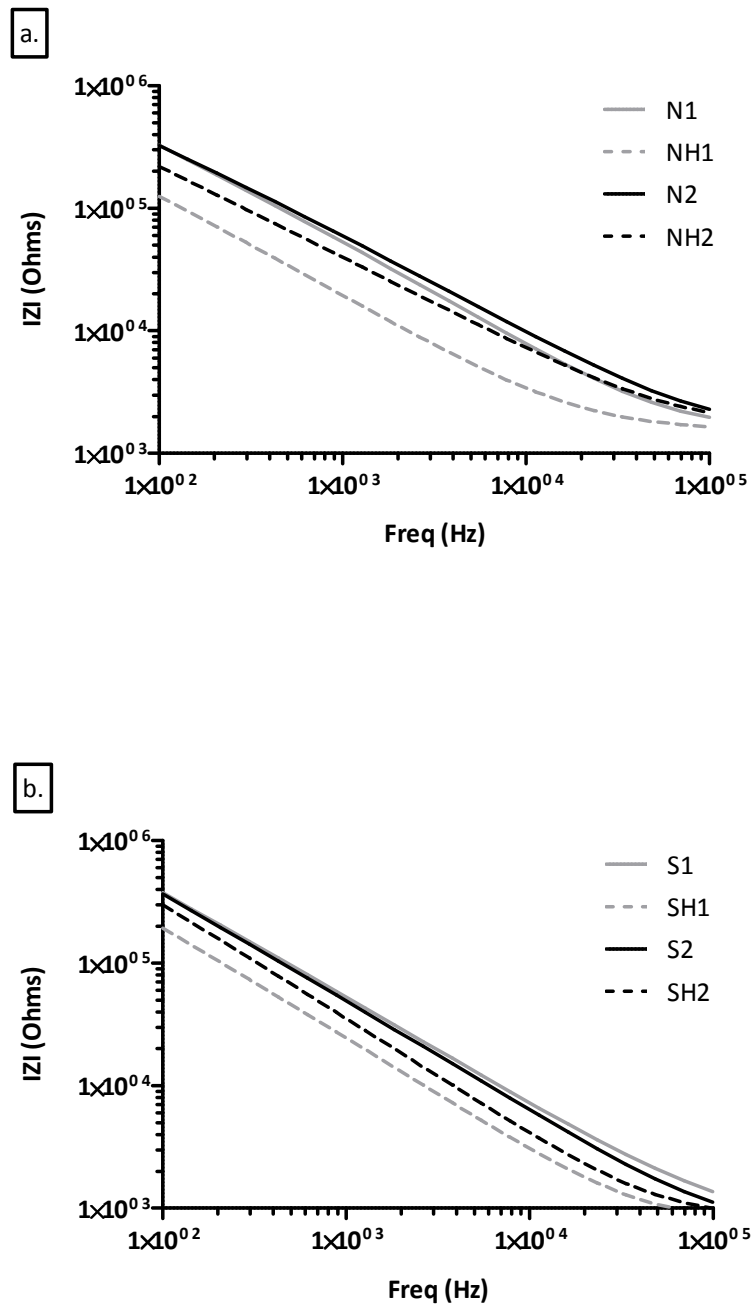


Figure 4.8. Evolution of the impedance modulus as a function of frequency.

(a) micro-needles (series N) and (b) planar-sensors (series S) with pHEMA (letter H). The difference between 100 nm (1) and 200 nm (2) is represented in the graphics.

The latter experiment repeated using sensors coated with black platinum in order to confirm the same behavior in platinated electrodes. Figure 4.9. shows the impedance results once the micro-needles (N) and the planar-sensors (S) are electroplated and coated with 100 nm of pHEMA film. The graphics also confirms the behavior of the pHEMA with the impedance sensors treated with black platinum.

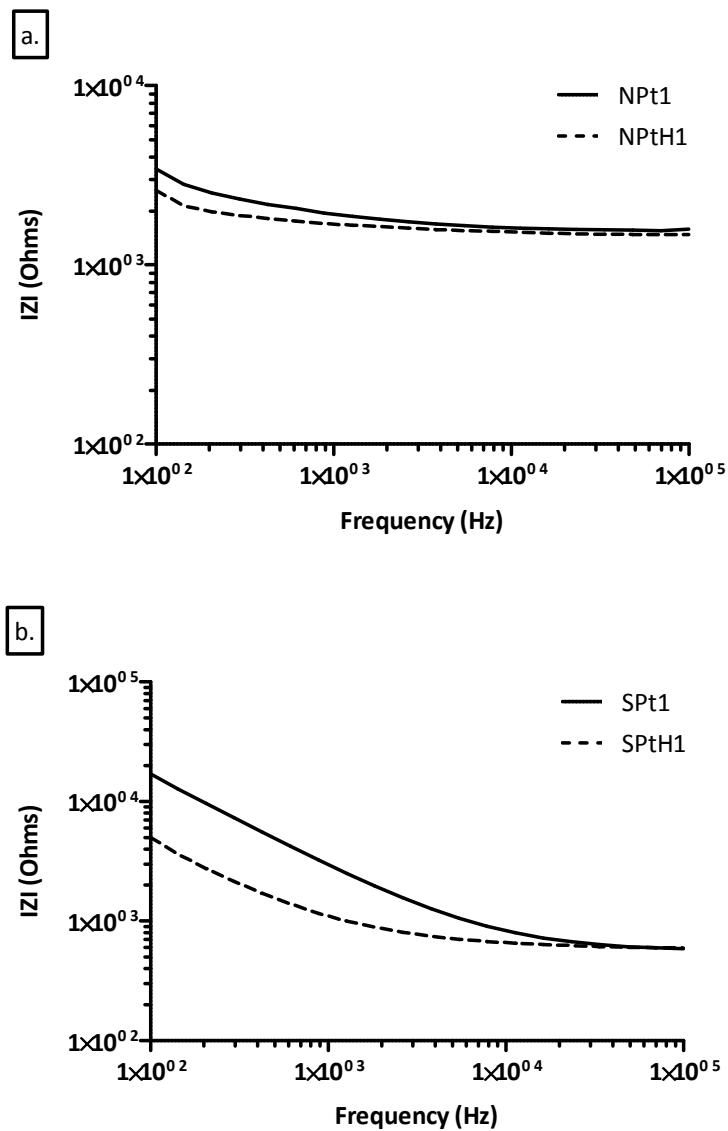


Figure 4.9. Evolution of the impedance modulus as a function of frequency.

(a) micro-needles (series N) and (b) planar-sensors (series S) coated with pHEMA (letter H) and without pHEMA. All sensors were electroplated (Pt).

4.3.3. Mechanical Characterization of Sensors

Once the device has been favorable electrically tested and validated for their experimental use, it is necessary to demonstrate that the sensor with the pHEMA coating is stable and robust. Usually, the biomedical devices can be in contact with tissue or have to withstand forces to be able to penetrate biological tissue. In order to demonstrate that pHEMA is mechanically resistant several simple, but harsh mechanical procedures, consisting of repeated insertions and extractions of the device in a foam layer and a vibration with a sonication in an ultrasonic bath were performed. The evolution of the detachment of the fragile black-platinum was followed by impedance measurements performed in the influence region of the Cd_i (100 Hz), as shown in Figure 4.10. Two sensors were used in this study, one coated (lines) with pHEMA and one uncoated (squares). As seen before, the initial impedance values of the bare gold electrodes have high values (gold) that drastically decrease with the electrodeposition of black platinum (black Pt). Interestingly, the impedance standard deviation of black platinum coatings is higher than the bare gold electrodes. This different behavior can be explained by the different RMS roughness values mentioned before ($RMS_{Gold} = 7 \pm 3$ nm; $RMS_{Black Pt} = 251 \pm 117$ nm). As observed before, pHEMA deposition results in a slight decrease of its impedance value (pHEMA). The sensor with pHEMA keeps its impedance unaltered after the application of ultrasounds and after the abrasion produced by the contact with a foam layer. However, the same sensor with black platinum and without pHEMA presents a severe increase in the first treatment and the second one nearly reached initial impedance values, which indicate the total detachment of the black platinum. These results were confirmed after observation of the electrodes by optical microscopy

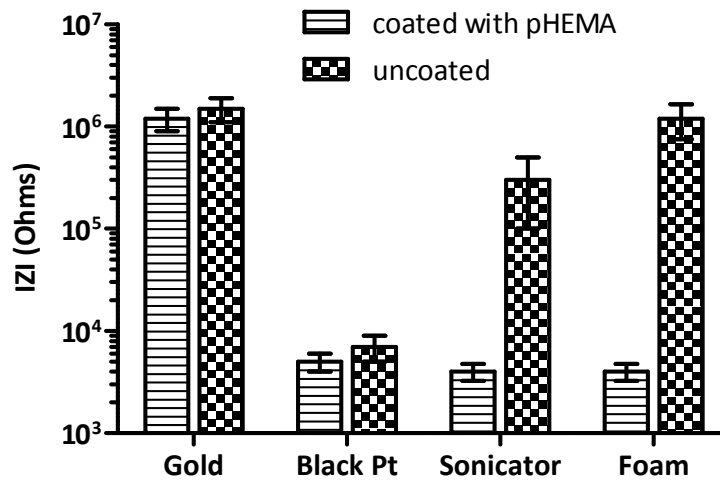


Figure 4.10. Evolution of the impedance module at 100 Hz of sensors coated (lines) and uncoated (squares) in saline solution.

The difference for bare electrodes, modified with black platinum, applying ultrasounds (sonicator) and scratching with a foam layer (foam) is represented.

The pHEMA coating was tested in an *in vivo* experiment with a real tissue contact to verify the promising *in vitro* results. In Figure 4.11., the electrode-electrolyte impedance evolution in saline solution of the electrodes for 10 experiments is shown. In each experiment, the electrodes of the device were in direct contact with the rabbit cornea (for both eyes) 8 times per eye and then the impedance was registered in saline solution to determine the electrode state. Two devices (one for each eye), each one having 8 electrodes were used, hence a total of 16 measurements were obtained and their average mean together with the standard deviation was represented for each experimental point in the error bars. Clearly, the changes in impedance after the second rabbit demonstrate that black platinum is only stable for the first cornea contact. Valid impedance values were not reached in this second experiment and required a sensor change.

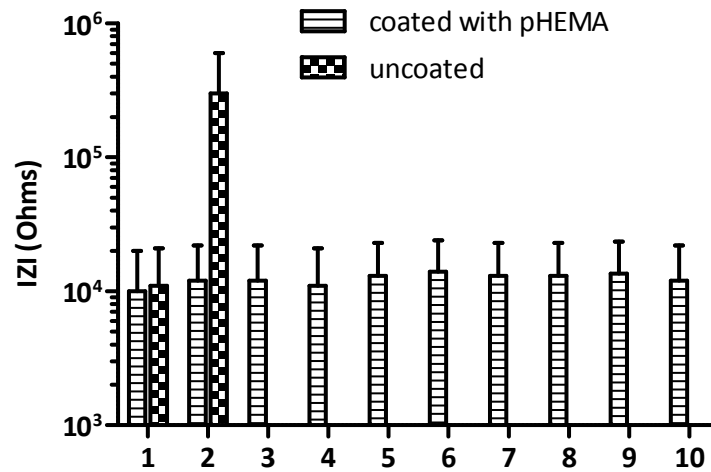


Figure 4.11. 10 *in vivo* experiments after getting the sensor in contact with a rabbit cornea. Evolution of the impedance module at 100 Hz of sensors coated (lines) and uncoated (squares) in saline solution.

The released black platinum could be visually as observed in the highlighted area in Figure 4.12. In order to maintain the biocompatibility of the biomedical device, in any *in vivo* application, the loss of material should be limited.

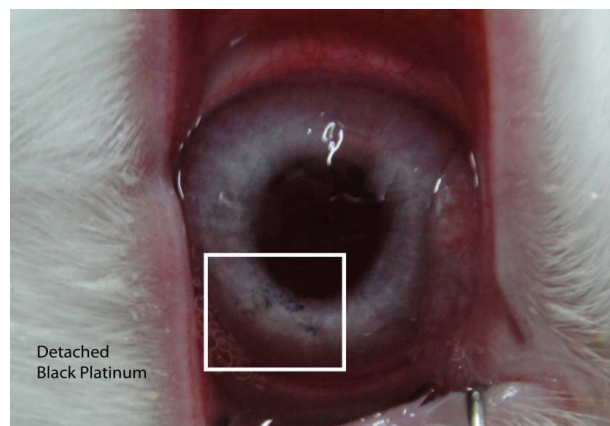


Figure 4.12. Picture taken in *in vivo* experiments to demonstrate the black platinum detachment for uncoated device.

In contrast, the use of pHEMA on the electrode surface makes the impedance to remain nearly unaltered overall the experimental (nearly 160 contacts with the tissue), allowing a better

repeatability and reproducibility of the *in vivo* work. These results show the excellent mechanical stability provided by the pHEMA accomplished with the pHEMA accomplished by coating the electrodes with pHEMA hydrogel. Thus, the lifetime of the microelectrode array system can be widely extended as the result of the stabilization of the platinum black layer. Hence, pHEMA coatings have the potential to improve the recordings of any applications that require contact between tissue and electrode.

4.4. Discussion

A new method to increase the mechanical stability of electrodeposited black platinum on biosensors has been presented here. The thin hydrogel layer of pHEMA deposited by iCVD allows the passage of the analytes and is conformal to the black platinum coating. Moreover the conformal recovery of the pHEMA hydrogel, which easily swells, could favor the hydration process increasing at the same time the contact area, which would slightly decrease the impedance module of the sensor, improving the sensor performance.

This work has demonstrated that iCVD is a gentle method for preparing stable, reversibly swellable films with nanoscale mesh size. The protective layer improves the impedance results observing a decrease in the signal even without black platinum. It has shown that the hydrogel deposited on the sensors with black platinum protects them against mechanical abrasion.

The hydrogel enhances the surface resistance of the post-processed microelectrodes. pHEMA does significantly not alter the electrode impedance because the mesh size is small enough to allow the diffusion of the electrolyte. The hydrogel deposited is chemically inert and protects the black platinum layer against mechanical damage. It has been demonstrated that the thickness of the deposited pHEMA hydrogel plays an important role in the impedance measurement. In this sense, the diffusion and hydration process is more pronounced with a thickness of 100 nm.

Stability of the pHEMA microelectrodes has been test mechanically and electrically in-vitro and in-vivo experiments. Also, its biocompatibility has been demonstrated, allowing a continuous use of the biosensor in contact with living tissues. This results in a better repeatability and

reproducibility within the *in vivo* experiments and has the potential to greatly improve the performance of implantable electrodes for short and long term studies.

Because of the presence of bacteria in biological environments and the importance of bacterial attachment in the biosensors presented, the next logical step is to study a protectable surface with the potential to resist biofilm formation.

Chapter 5.

Thermo-responsive Behavior of pNIPAAm

Thin Film Hydrogels by QCM-D

5.1. Introduction

The last part of this thesis was centered in the study of the iCVD techniques described earlier to develop temperature responsive hydrogels, covering a range of transition temperatures. This chapter also reports the use of quartz microbalance techniques to allow characterization of temperature responsive hydrogels and monitoring of the temperature-driven changes in the hydrogel state. Thermo-responsive hydrogels offer the opportunity to develop a new interface between bio-device and media that may contain microorganism.

Infection of medical devices is a life threatening complication and can lead to significant problems. Therefore, it is of great importance to enhance the antibacterial properties of polymers (Nichols & Raad 1999; Vuong & Otto 2002; Akovali et al. 1998). It has been reported that the antibacterial properties of surfaces can be improved by the deposition of surface coatings containing antimicrobial agents or by the incorporation of biocide agents into bulk polymers (Jones et al. 2005; Yuan et al. 2004). The main disadvantage of these approaches is that the antimicrobial properties are stable during a limited period of time. Bio-devices are now being designed to have long life-times; therefore better strategies are needed in order to face microorganism-related problems, such as colonization and or biofilm formation.

Stimuli-responsive polymers change their physicochemical properties, often the strength of the interaction with water (wettability), in response to changes in the environmental conditions. This sensitive hydrogels, swell or collapse in response to external changes such as pH (Geismann & Ulbricht 2005; Iwata et al. 1998), temperature (Alf et al. 2011; Spohr et al. 1998; Lokuge et al. 2006), light (Deshmukh et al. 2009; Y. S. Park et al. 1998) or electric field (Giacomelli et al. 2010; Böker et al. 2003). The development of responsive and smart surfaces

has been extensively investigated (Adiga & Brenner 2012; Aguilar et al. 2007) and makes them suitable for microelectromechanical systems, especially for biological applications, such as sensor and biomedical applications (Stuart et al. 2010). One of the most intensively studied stimuli-responsive polymers is the thermo-sensitive polymer.

Thermo-sensitive polymers can be defined as polymers that, when dissolved in aqueous solutions, undergo a transition to gel state in response to changing temperature. Such a structural transition may have a number of possible applications where a responsive character triggered by temperature is really interesting, including drug delivery systems (Abulateefeh et al. 2011; Zhang et al. 2002), permeation filters (W. Song et al. 2007; Chu et al. 2011), attachment-switchable surfaces for biomaterials (Cole et al. 2009; Ista et al. 2010) and functional composite surfaces (Alam et al. 2007). Thermo-sensitive hydrogels dramatically change their swelling degree in water above or below a certain temperature, known as lower critical solution temperature (LCST) or upper critical solution temperature (UCST) respectively (Tian et al. 2011; Schmaljohann 2006).

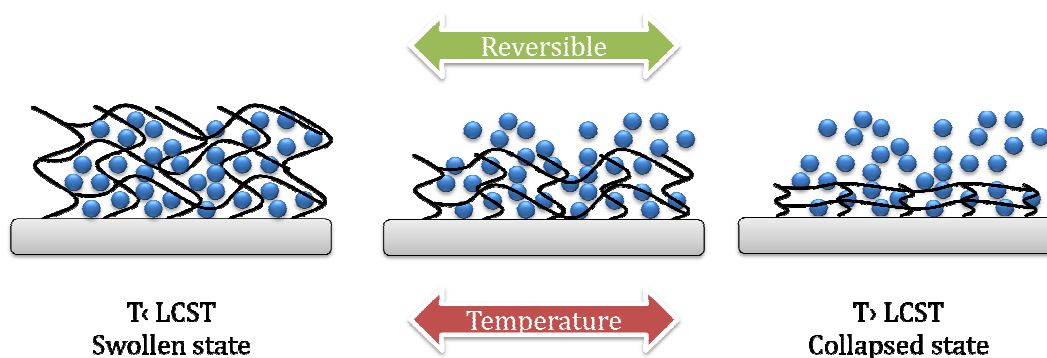


Figure 5.1. Schematic representation of the internal transition of thermo-responsive films. Thermo-sensitive hydrogel presents a swollen state below the LCST and a collapsed state when the temperature rises above the LCST. The phase transition of this hydrogels is reversible.

Poly(*N*-isopropylacrylamide) (pNIPAAm), a polymer that undergoes a dramatic change in hydrophobicity/hydrophilicity balance with temperature, is the principle most typical example of a responsive polymer (Ishida & Biggs 2007; E. J. Park et al. 2007). Its behavior in solution is well-known and has been studied extensively. pNIPAAm exhibits a LCST near 32 °C, below this temperature, the polymer chains are fully hydrated in solution, however above the LCST the polymer chains undergo a sharp change in hydrophobicity and collapse into a globular state. Below the LCST, the hydrophilic amide groups of NIPAAm making the polymer soluble. Above the LCST, hydrogen bonding occurs both inter- and intra-chain between amide groups, thus exposing the hydrophobic isopropyl group to the solution and causing phase segregation (Urban 2011). The introduction of hydrophilic or hydrophobic co-monomers in pNIPAAm polymers enables tuning of the LCST.

It is possible to synthesize temperature-sensitive hydrogels by introducing appropriate monomers having thermo-sensitive properties in the hydrogel structure. These hydrogels are generally obtained by the co-polymerization of three main components: two co-monomers that generally modulate the thermo-sensitivity of the hydrogel and a cross-linker. This thermo-responsive behavior and the ability to make pNIPAAm based hydrogels have led the possibility to modulate the desired properties depending on the application.

Recently, interest has grown in fabricating thin films based on pNIPAAm because the main applications have micro- or nano-structure (Adiga et al. 2008; Tokarev & Minko 2009). Many techniques have been described to provide surface modification, such as surface-initiated polymerization, pNIPAAm-end functionalized (Cunliffe et al. 2003; Ma et al. 2009) and vapor-phase methods (CVD) that include iCVD (Alf et al. 2011; Ozaydin Ince et al. 2011). In order to use thermo-sensitive hydrogels in a given application with a desired temperature-responsive,

it is important to characterize the LCST appropriately. Due to the increasing concern to understand this property of the films, a variety of analytical tools, including XPS (Pan et al. 2010), AFM (Zareie et al. 2000), WCA (Zoppe et al. 2012) and QCM-D (Ishida & Biggs 2007) have been developed.

Regarding the importance to define the LCST, the present work is focused in the study of pNIPAAm based hydrogels by Quartz Crystal Microbalance with Dissipation (Jhon et al. 2006; Laloyaux et al. 2010; Olsson et al. 2009). QCM-D monitors the frequency and energy dissipation response of a freely oscillating sensor, giving a faster and accurate information of the thin films attached to the sensors. Ever since Sauerbrey (Sauerbrey 1959) determined the relationship between the mass change per unit area at the QCM-D electrode surface and the observed change in oscillation frequency of the crystal, many coatings have been characterized using traditional QCM. More recently, due to the Sauerbrey's limitations, the equation is only applicable to uniform and rigid thin films, scientists centered their studies in extend the applications for this technique. They realized that it can be used in contact with liquids and viscoelastic property can be taken into account with dissipation technique. This technique should be in good agreement with the measurements provided in oscillation frequency. In this case, the crystal is driven at its resonant frequency by an oscillator that can be intermittently disconnected causing the crystal oscillation amplitude to decay (Gamry Instruments 2012). This technique is presented as a feasible platform to analyze and define the LCST property to obtain the desired temperature for each application.

In this chapter, a library of temperature responsive hydrogels has been generated to cover a range of different LCST and QCM-D analysis (frequency and dissipation) depending on the hydrogel behavior application. Besides, in the literature is possible to find a great number of

applications related with bio-medical applications, and the adhesion of bacteria on the surfaces of materials is crucial in contamination of bio-devices. Therefore, the use of temperature responsive hydrogels to develop a surface that can reduce bacteria adhesion thanks to the topography changes and at the same time can embed and deliver an antibacterial substance due to the swell and collapse state.

5.2. Experimental Section

5.2.1. Synthesis of Thermo-responsive Films via iCVD

Different experimental series were performed to synthesize and characterize pNIPAAm copolymer thin films in order to study the possibility to achieve tunable LCSTs and to improve the antimicrobial behavior of surfaces in contact with microorganism.

All reactions were conducted in a custom- built reactor as described earlier (see 2.2.1 or 3.2.1.) N-isopropylacrylamide (NIPAAm, Acros Organics, 99%), Acrylic acid (AAc, Sigma Aldrich, 99%), N,N-diethyl acrylamide (DEAAm, Polyscience, 95%), N,N-dimethyl acrylamide (DMAAm, Sigma Aldrich, 99%), Ethylene glycol diacrylate (EGDA, Sigma Aldrich, 90%) as a cross-linker and tert-Butyl peroxide (TBPO, Sigma Aldrich,98%) as an initiator, were purchased and used without further purification.

To deposit the iCVD films, TBPO vapors were used at room temperature and fed into the reaction chamber through a needle valve to control the pressure of the initiator. The monomers NIPAAm and EGDA were kept at 75 °C and 65 °C respectively and fed into the reactor via a needle valve to control and modify the NIPAAm flow rate between samples. In comparison with NIPAAm films, where only NIPAAm, EGDA and TBPO were fed into to reactor,

in NIPAAm copolymers, DMAAm, DEAAm were kept at 85 °C and AAc at 75 °C. The vapors were fed into the reactor via a needle valve to obtain the desired flow rate.

The reactor was equipped with a filament array, which provided thermal energy for selective decomposition of molecules. The clearance between the filaments and the stage was 29 mm. The Nichrome filaments (80% Ni/20% Cr, AWG 26, Omega Engineering) were resistively heated to 200 °C, as measured by a thermocouple (Type K, AWG 36, Omega Engineering) directly attached to one of them. The monomers and initiator streams were joined before entering the reactor, once the streams reached the chamber reactor, the polymerization took place all over the chamber.

The substrate was maintained at a temperature of 35 °C to promote monomer adsorption and the film growth was terminated at approximately 50 nm. The final pressure in each deposition was 1.5 Torr. For samples used in subsequent characterization, both QCM-D gold-coated crystals with a fundamental frequency of 5 MHz (QSX-301, Q-sense) and bare Si wafers (WaerWorld) were included as substrates during depositions. In order to identify the LCST of the coatings and how it varies between them, four experimental settings were conducted.

Table 5.1. Experimental conditions for all the film synthesis.

Series A, NIPAAm thin films, no copolymer were used during the deposition. Series B, NIPAAm-co-DMAAm, series C, NIPAAm-co-AAc, and series D, DEEAAm-co-DMAAm.

Sample Name	NIPAAm rate (%)	DEEAAm rate (%)	EGDA rate (%)	DMAAm rate (%)	AAc rate (%)
A1	90	-	10	-	-
A2	80	-	20	-	-
A3	70	-	30	-	-
B1	80	-	10	10	-
B2	70	-	10	20	-
B3	60	-	10	30	-
C1	80	-	10	-	10
C2	70	-	10	-	20
C3	60	-	10	-	30
D1	-	80	10	10	-
D2	-	70	10	20	-
D3	-	60	10	30	-

TBPO does not have influence in the final LCST so the flow was maintained constant for all deposition, and the final flow-rate is calculated without its fraction (Chan & Gleason 2005a).

5.2.2. Thermo-responsive Surface Characterization

Water Contact Angle (WCA)

Contact goniometry was used in order to determine the wettability of the thin film thermo-responsive hydrogels when the temperature is above or below the LCST. The term “wetting” describes the contact between a liquid and a solid surface and is a result of intermolecular interactions when the two are brought together. The amount of wetting depends on the energies (or surface tensions) of the interfaces involved. The degree of wetting is described by

the contact angle. This is the angle at which the liquid/vapor interface meets the solid/liquid interface.

The contact angle was measured from a drop of PBS buffer solution resting on the thin film. If the liquid is very strongly attracted to the solid surface (hydrophilic surfaces), the droplet will spread out on the solid surface and the contact angle will present values comprised between zero and 90° . When the temperature was below LCST, the hydrogel swells and the result was a hydrophilic coating. If the solid surface is hydrophobic, the contact angle will be larger than 90° , such as occurs at higher temperature when the surfaces collapse.

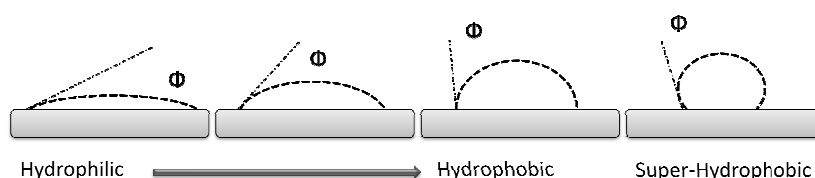


Figure 5.2. Schematic result of a WCA experiment(Krüss n.d.)

Contact angle measurements were determined on a goniometer (DSA 100, Krüss). Si wafer was cut into 25 x 25 mm strips prior the measuring and placed over a home-built heater plate. Measurements were taken both at room temperature (25°C) and after heating the substrate (50°C) with the heating element. $5\ \mu\text{L}$ of PBS was dropped using a dispenser mounted on the goniometer, PBS was first heated for the high temperature experiment.

Atomic Force Microscope (AFM)

AFM studies were performed using an XE-100 Atomic Force Microscope (Park Scientific Instruments), with lateral resolution of 0.15 nm and vertical resolution of 0.05 nm, in non-