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Bioelectrochemistry 62 (2004) 19–27
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

# Adhesion and proliferation of cells on new polymers modified biomaterials

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Received 21 March 2003; received in revised form 11 September 2003; accepted 26 September 2003

**Abstract**

Up to today, several techniques have been used to maintain cells in culture for studying many aspects of cell biology and physiology. More often, cell culture is dependent on proper anchorage of cells to the growth surface. Poly-L-lysine is commonly used as adhesive molecule. In this study, we present, as an alternative to poly-L-lysine, new polymer film substrates, realized by electropolymerization of different monomers on fluorine-doped tin oxide (FTO) surfaces since electropolymerization is a good method to coat selectively metallic or semiconducting electrodes with polymer films. So, the adhesion, proliferation and morphology of rat neuronal cell lines were investigated on polymer treated surfaces. Several amine-based biocompatible polymers were tested: polyethyleneimine (PEI), polypropyleneimine (PPI), polypyrrole (PPy) and poly(*p*-phenylenediamine) (PPPD). These polymer films were coated on FTO surfaces by electrochemical oxidation. After 8 h in a culture medium, a high percentage of cells was found to be attached to PEI and PPI compared to the other polymers and to the reference surfaces (glass and FTO uncovered). After 24 and 72 h in the culture medium, cells were found to proliferate faster on PEI and PPI than on other polymers and reference surfaces. Consequently, cells have a greater fold expansion on PEI and PPI than on PPPD, PPy or glass and FTO uncoated. From these results, we deduce that PEI and PPI can be useful as coating surface to cultivate neuronal cells.
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*Keywords:* Neuronal cell lines; Cell adhesion and proliferation; Cell culture; Electrochemistry; Polymers

## 1. Introduction

In recent years, there has been a growing interest in electrochemistry as a tool for biological and biomedical applications. Thus, some fields of research are intensively studied, in particular the electrical or magnetic effects on the development of cells [1–3], the electrochemotherapy [4,5], the development of ultramicroelectrodes studying cell mechanisms [6] or the search for new culture substrates [7]. Indeed, selective surface modification is a necessary step to direct cellular adhesion and growth, since chemical surface modification [8,9], topographical surface modifications [10,11] or both [12,13] are commonly used. The factors that are believed to play an essential role in cell adhesion mechanisms are: specific surface chemistry [14], surface hydrophobicity [15], topography [16], surface charge [17] and protein interactions [18].

Several materials with original processing techniques have been proposed for being used in cell culture. A frequently reported non-specific approach is the chemical surface modification of silica-like materials with silanes, alkylsilanes and amino-rich alkoxylanes, which produce either non-adhesive or adhesive substrates [19]. Unfortunately, a general acknowledged problem of silane modified surfaces is the hydrolysis of the Si—O—Si bonds, which occurs under physiological conditions. A silane-based system able to maintain cellular patterns over long periods of time is therefore unlikely and has not been reported yet. Biomolecules represent another alternative to study cellular patterns on surfaces. Extracellular matrix (ECM) components like fibronectin or laminin [20,21] are very effective as

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doi:10.1016/j.bioelechem.2003.09.009

cell-adhesive substances. In contrast, albumin is an effective non-adhesive biological coating on hydrophobic surfaces due to the irreversible strong adsorption and conformational change [22]. However, our present work let us thinking that polymers are a better alternative to other current biomaterials commonly used. Indeed, polymers we tested are cheap, easy to synthesize, biodegradable and biocompatible [23–25]. This confirms previous studies, which have shown that electrosynthesized polymers used as biomaterials can be more efficient than biological polymers such as poly-L-lysine [26–29]. Indeed, Bledi et al. [26] used control plates pretreated with poly-L-lysine and compared them to other plates coated with polyethyleneimine (PEI). They showed that no difference appears during five days after the plating. However, after 10 days of differentiation, the cells were uniformly spread and interconnected by extensive network of axons and dendrites on PEI but not on poly-L-lysine. More cells formed clumps sensitive to washing on poly-L-lysine, consequently their detachment was unavoidable. We must also discuss about the stability in time of biological molecules since it is a crucial problem. For example, Frödjdö et al. [27] showed that cells attach and start to grow on all biomaterials. However, the proliferation was favored on double-coated surfaces (lysine/laminin or poly-L-lysine/laminin). Recently, Blau et al. [29] used polymers coupled to biological molecules to improve cell adhesion. They showed that the effectiveness of laminin or poly-L-lysine depends on the addition of serum in the medium and on the type of the substrate. In contrast, the adhesion promoting of synthesized polymer films seems to be based on more direct cell–film interactions with the film masking the substrate properties.

Consequently, our work consisted in coating polymeric films onto surfaces by an original electrochemical way [30,31] in order to favour cells growth on these polymers. So, we performed electrochemical oxidations of different monomers such as ethylenediamine (EDA), pyrrole (Py), diethylenetriamine (DETA) and 1,3-diaminopropane (1,3-DAP) to electrodeposit polymeric films at working electrode surfaces. We used FTO as working electrode since this semiconductor is optically transparent in the visible spectrum range and allows anodic oxidation of the monomers. Then, we used infrared spectroscopy to identify the electrodeposited polymer films. For instance, it has already been proved in previous works that anodic oxidation of EDA or DETA leads to a linear film of linear polyethyleneimine, that 1,3-DAP leads to linear polypropyleneimine [32,33], pyrrole to polypyrrole (PPy) [34] and *p*-phenylenediamine to poly(*p*-phenylenediamine) (PPPD) [35] on platinum surfaces.

Then, we tested the capacity of these different polymers to improve cells adhesion and proliferation. We used a rat neuronal cell line to check the adhesion and the proliferation of these cells. So we plated neuronal cells in contact with several FTO passivated surfaces, coated with the different polymers, in a culture medium. Then, we let the olfactory cells adhere and, during their growth, we observed the changes in their morphology using a confocal microscope since FTO is a transparent surface, which allowed us to follow directly the development of our cells. Thanks to this qualitative observation, we can conclude that the development of the cells is normal. Note that we did not realize immunostain essay since we did not want to differentiate neurons from glia astrocyte or oligodendrocyte, which compose cell lines, but we only wanted to demonstrate cells adhere on the substrate.

So our electrodeposited polymers seemed to be biocompatible and they allowed cells to adhere and proliferate. More, the adhesion rate (which corresponding to the number of cells plated on the surface 8 h after their seeding in the culture medium divided by the number of cells introduced in the culture medium) and the proliferation rates (which are the number of cells on the surface 24 and 72 h after their seeding in the culture medium divided by the number of cells introduced) were calculated for each polymer in order to quantify and compare the ability of the olfactory cells to adhere and proliferate on the different polymers. These rates indicated that PEI and polypropyleneimine (PPI) are the best polymers to cultivate olfactory cells since their adhesion and proliferation rates are far much higher than those obtained for PPy, poly(*p*-phenylenediamine) (PPPD) or uncovered FTO and glass. So we demonstrated that a FTO surface coated with PEI or PPI may be a good substrate for the culture of olfactory cells.

## 2. Materials and methods

### 2.1. Biochemical procedures

#### 2.1.1. Reagents

We used a rat neuronal cell line 13S124 transformed by $E_1A$ antigen from an adenovirus. Cells were harvested and cultivated in a Petri dish (diameter: 50 mm) for 8, 24 or 72 h. Cells were cultivated in DMEM media with 10% of fetal calf serum (from Gibco). All media were supplemented with 200 mM of L-glutamine and antibiotics: penicillin (100 U/ml) and streptomycin (10 μg/ml). After 8, 24 and 72 h, the cells were treated with trypsin 2.5% (tenfold dilute in HBSS $Ca^{2+}$- and $Mg^{2+}$-free) during 10 min, 37 °C. Trypan blue exclusion test (0.4%) was used to discriminate and count living cells.

#### 2.1.2. Cell adhesion studies

To follow the development of the cells, we used a confocal microscope Olympus OM2. The cells were allowed to adhere to the substrates undisturbed in a humidified incubator (at 37 °C, 5% $CO_2$) for 8 h. We tested three samples in parallel for each polymer. The number of plated cells was quantified by counting with an hematocytometer. The cells were seeded on each polymer at the same density of cells for each sample and for each polymer and then incubated for 8 h.

*2.1.3. Cell proliferation studies*

We introduced in the Petri dish $2.10^5$ cells/ml. Indeed, total cell count was performed using trypan blue staining method with a hemacytometer and the cell density was then adjusted to about $2.10^5$ cells/ml and the volume of the cell suspension used is 100 μl. Thus, these cells can adhere and proliferate onto polymer coated FTO. Cells were allowed to attach and proliferate for 24 and 72 h at which time point the number of plated cells was determined by counting with an hematocytometer. We do not pursue the culture after 72 h because confluence was reached after 90 h in the culture medium.

*2.2. Chemical procedures*

*2.2.1. Reagents*

All the electrolytes were obtained from Aldrich (analytical grade) and lithium trifluoromethanesulfonimide, LiTFSI, was from Fluka. The electrolytes (EDA, DETA, 1,3-DAP, pyrrole and *p*-phenylenediamine) and the lithium salt were used as received. LiTFSI was used as a supporting electrolyte at a concentration of $10^{-3}$ M, and was added to EDA, 1,3-DAP and DETA in a glove box from Jacomex under a dehydrated Ar atmosphere. PPD was used at the concentration of 0.1 M in a phosphate buffer solution (pH = 7 for this solution), pyrrole was used at the concentration of 0.1 M in a solution of KCl at a concentration of 0.1M (pH = 7 for this solution).

*2.2.2. Apparatus and methods*

The electrochemical apparatus was a classical three-electrode setup using an Autolab PGSTAT 20, from Ecochemie, potentiostat-galvanostat. The working electrode was a FTO surface of 3.7 $cm^2$ (from Balzers, 10 $\Omega/cm^2$). The reference electrode was a silver wire noted as Silver Reference Electrode (SRE), the counter-electrode was a platinum wire. All electrochemical experiments were carried out at room temperature (293 K) with a sweep rate of 20 mV/s between 0 and +4 V/SRE and with a hold at +4 V/SRE during the first scan. Each solution was purged by ultrahigh purity argon. We used a NEXUS 470 spectrophotometer coupled to an Omnic software from Nicolet to perform infrared-attenuated total reflection (IR-ATR) spectra. After electropolymerization and just before cells were seeded, FTO was washed in absolute ethanol during 15 min. Polymers used cover approximately 2.4 $cm^2$ of each FTO surface.

## 3. Results

*3.1. Electrodeposition of the polymer films*

Electrodeposited poly(*p*-phenylenediamine) aroused interest in the last few years in biosensor applications [36]. Fig. 1a shows the cyclic voltammogram of PPD, swept in pH = 7.0 phosphate buffer solution containing 0.1 M PPD, from 0 to +4 V/SRE at 20 mV/s at a FTO electrode. It can easily be noticed that the electrochemical oxidation of PPD was completely irreversible and that the oxidation peak can be observed at a potential of +1.7 V/SRE. Oxidation current of PPD dropped during successive steps and, simultaneously, there is a decrease of the potential corresponding to the oxidation peak. This behaviour is indicative of the formation of a non-conductive polymeric film on the electrode surface. *p*-Phenylenediamine, as the isomer of *o*-phenylenediamine, contains two amino groups in its molecular structure. It can be oxidized at a positive potential to produce PPPD throughout an ECE mechanism [36–39].

Then, we wanted to study the anodic oxidation of an electrolyte composed with one salt, LiTFSI, dissolved in 1,3-DAP. Thus, for 1,3-DAP and at a FTO electrode, an anodic peak was observed at +2.2 V/SRE during the first scan (Fig. 1b). For subsequent scans, there was a drastic drop of current. So the anodic oxidation of 1,3-DAP leads to the formation of a passivating film at the electrode surface. The peak broadness is characteristic of primary aliphatic amines and renders the experimental study of the electrochemical mechanism impossible. The same general trends can be observed for the oxidation of DETA and EDA at FTO surfaces. Indeed, during the first scan: two peaks appeared at +1.9 and +3.1 V/SRE for DETA-based-electrolytes and one peak at +2.7 V/SRE for EDA-based-electrolytes. During the second scan and for the following ones, it gave a drop of current and all the peaks have disappeared.

Since EDA and 1,3-DAP contain two amino groups and DETA three amino groups (two primary and one secondary) in their molecular structure, they can easily be oxidized at a positive potential to produce cationic radicals and further react with other monomers to form polyethyleneimine from EDA and DETA or polypropyleneimine from 1,3-DAP. The mechanisms of these electropolymerizations have recently been established using ab initio calculations at the Hartree-Fock level of theory [40,41]. The growth of these polyalkyleneimines described here can occur on different metallic or semiconducting electrodes: smooth gold, smooth platinum, glassy carbon, p- or n-type silicon [42].

Finally, the cyclic voltammogram of PPy at a concentration of 0.1 M swept in a KCl solution at a concentration of 0.1 M (pH = 7 for this solution) in the potential range going from 0 to +4 V/SRE at 20 mV/s at a FTO electrode was done. We can observe an oxidation peak at a potential of +2.3 V/SRE. This peak can be observed for all cycles. So the polymeric film formed at the FTO surface is a conductive one [34].

*3.2. Characterization of the polymeric films*

To determine the nature of the deposited films, we performed IR-ATR spectra from the films obtained by anodic

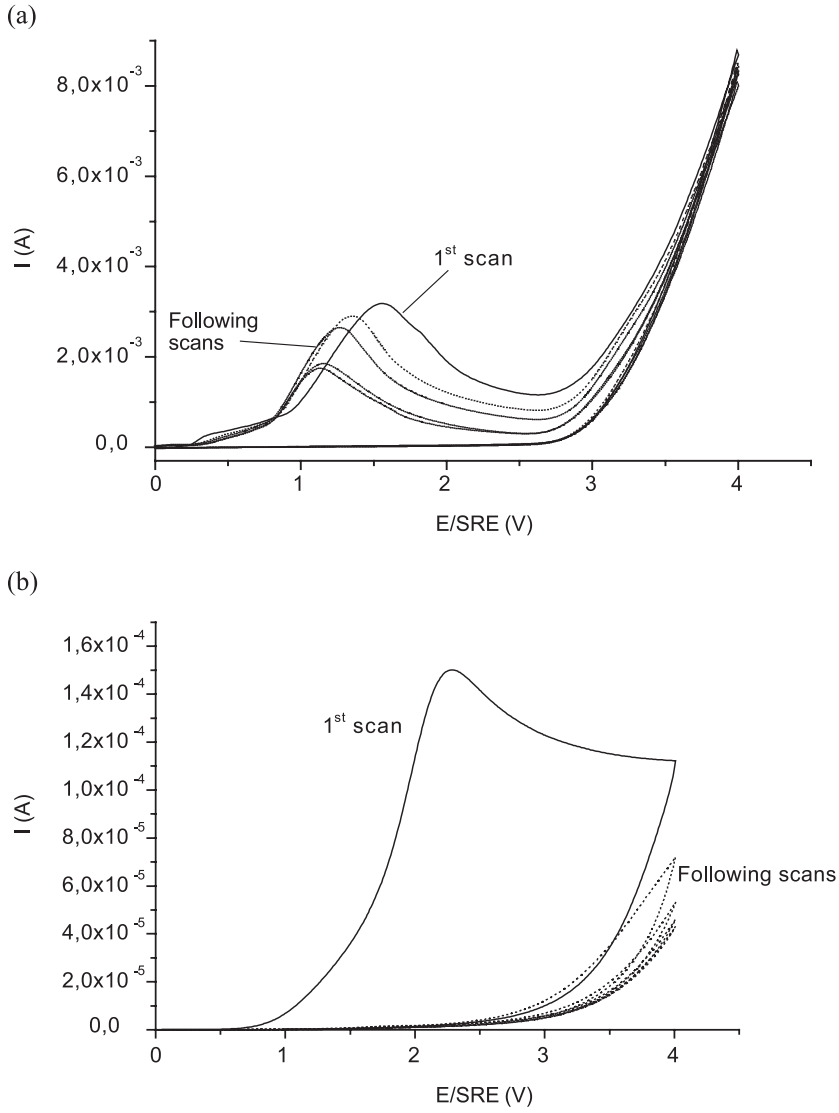



Fig. 1. (a) Cyclic voltammogram of PPD swept in pH = 7 phosphate buffer solution containing 0.1 M of PPD with a sweep rate of 20 mV/s. (b) Cyclic voltammogram of 1,3-DAP + LiTFSI $10^{-3}$ M with a sweep rate of 20 mV/s.

oxidation of EDA, DETA, 1,3-DAP, PPD and Py at FTO surfaces. All the passivated electrodes were preliminary rinsed with bi-distilled water and dried at 30 °C for 24 h. The spectrum obtained for PPD is shown Fig. 2. Table 1 summarizes the vibrational bands observed on all these IR spectra. These spectra allowed us to identify the film obtained from the oxidation of EDA and DETA as polyethyleneimine, $-(NH-CH_2-CH_2)-_n$, since its main characteristic bands appear on these spectra: N–H stretching (3200–3300 $cm^{-1}$) and N–H deformation (1600 $cm^{-1}$) of secondary amine, C–N stretching (1100 $cm^{-1}$), C–H stretching (2930 and 2860 $cm^{-1}$) and C–H deformation (1500 $cm^{-1}$) of aliphatic $-CH_2-$. The same analysis was carried out for oxidized 1,3-DAP and the corresponding IR-ATR spectrum showed that the oxidation of pure 1,3-DAP leads to polypropyleneimine, $-(NH-CH_2-CH_2-CH_2)-_n$, as it has already been shown with platinum electrodes [42], since all the vibration bands that appear on the IR spectrum are those characteristic from propyleneimine. We have also made a spectrum of oxidized PPD and the vibration bands appearing on the spectrum are those characteristic of poly(*p*-phenylenediamine): N–H stretching of primary amine (3387 and 3334 $cm^{-1}$), N–H stretching (3200 $cm^{-1}$) and N–H deformation (1678 $cm^{-1}$) of secondary amine, C–N aromatic stretching (1283 and 1251 $cm^{-1}$), C–H aromatic stretching (3040 $cm^{-1}$) and C–H aromatic deformation (827 $cm^{-1}$).

Then, a similar analysis was carried out for oxidized Py and the IR-ATR spectrum showed that the oxidation of Py leads to polypyrrole since the following vibrational bands appear on the infrared spectrum: N–H stretching of secondary amine (3332 $cm^{-1}$), N–H deformation (1698 $cm^{-1}$) of secondary amine, C–N primary amine aromatic stretching (1350 and 1280 $cm^{-1}$), C–H aromatic stretch-

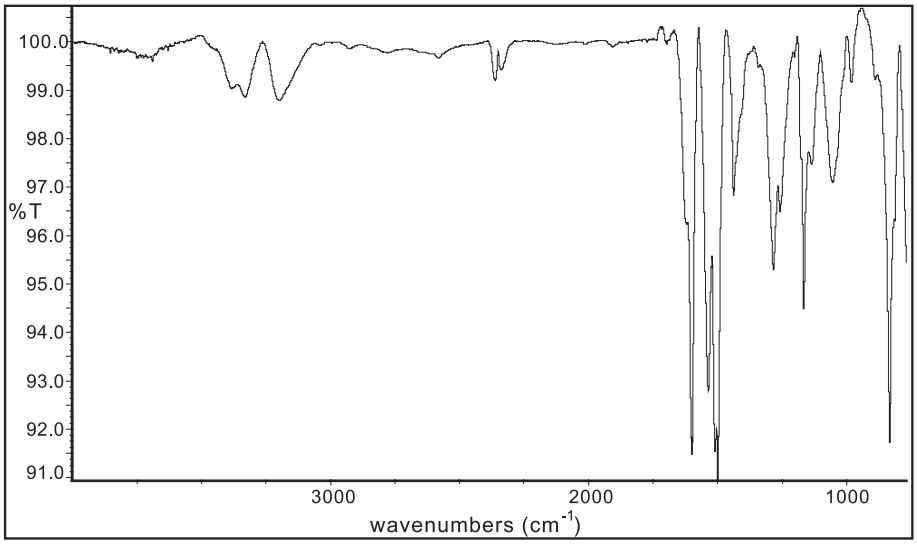



Fig. 2. IR-ATR spectrum of PPD swept in pH = 7 phosphate buffer solution containing 0.1 M of PPD with a sweep rate of 20 mV/s.

ing (3030 $cm^{-1}$), C–H aromatic deformation (700 and 880 $cm^{-1}$) and C–C aromatic stretching (1560 and 1447 $cm^{-1}$).

Consequently, we can assume that the oxidation of EDA, DETA, 1,3-DAP, PPD and Py at FTO surfaces lead to PEI, PEI, PPI, PPPD and PPy, respectively.

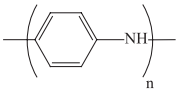
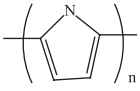
### 3.3. Cell culture

Our purpose is now to cultivate cells onto these polymers electrodeposited on FTO surfaces.

#### 3.3.1. Cells adhesion

First of all, we studied cell morphology using an inverted phase contrast microscope (Willovert S). We took photographs of the cells (Olympus OM2) 5, 8, 24 and 90 h after they have been seeded in the culture medium. We observed after 5 h in culture, that cells are able to adhere to the substrate (for instance onto PEI, see Fig. 3a). We observed some aggregates of cells, composed in vast majority by dead cells, which disappeared after a few hours when cells could no longer adhere to the substrate. Fortunately, these accumulations represent

Table 1
Vibrational bands of oxidized monomers

| Vibration mode | Oxidized EDA | Oxidized DETA | Oxidized 1,3-DAP | Oxidized PPD | Oxidized Py |
|---|---|---|---|---|---|
| N-H stretching secondary amine | 3350-3200 $cm^{-1}$ | 3200-3300 $cm^{-1}$ | 3300 $cm^{-1}$ | 3200 $cm^{-1}$ | 3332 $cm^{-1}$ |
| N-H deformation | 1569 $cm^{-1}$ | 1600 $cm^{-1}$ | 1570 $cm^{-1}$ | 1678 $cm^{-1}$ | 1698 $cm^{-1}$ |
| C-N stretching | 1162 $cm^{-1}$ | 1100 $cm^{-1}$ | 1125 $cm^{-1}$ | 1283 $cm^{-1}$<br>1251 $cm^{-1}$(aromatic) | 1350 $cm^{-1}$<br>1280 $cm^{-1}$(aromatic) |
| C-H asymmetric stretching | 2969 $cm^{-1}$<br>2926 $cm^{-1}$ | 2930 $cm^{-1}$<br>2860 $cm^{-1}$ | 2930 $cm^{-1}$<br>2880 $cm^{-1}$ | 3040 $cm^{-1}$<br>(aromatic) | 3030 $cm^{-1}$(aromatic) |
| C-H deformation | 1485 $cm^{-1}$<br>1285 $cm^{-1}$ | 1500 $cm^{-1}$ | 1490 $cm^{-1}$<br>1500 $cm^{-1}$ | 827 $cm^{-1}$(aromatic) | 700 $cm^{-1}$<br>800 $cm^{-1}$(aromatic) |
| C-C aromatic stretching | - | - | - | - | 1560 $cm^{-1}$<br>1447 $cm^{-1}$ |
| Polymer synthesized | $-(NH-CH_2-CH_2)-_n$ | $-(NH-CH_2-CH_2)-_n$ | $-(NH-(CH_2)_3)-_n$ |  |  |

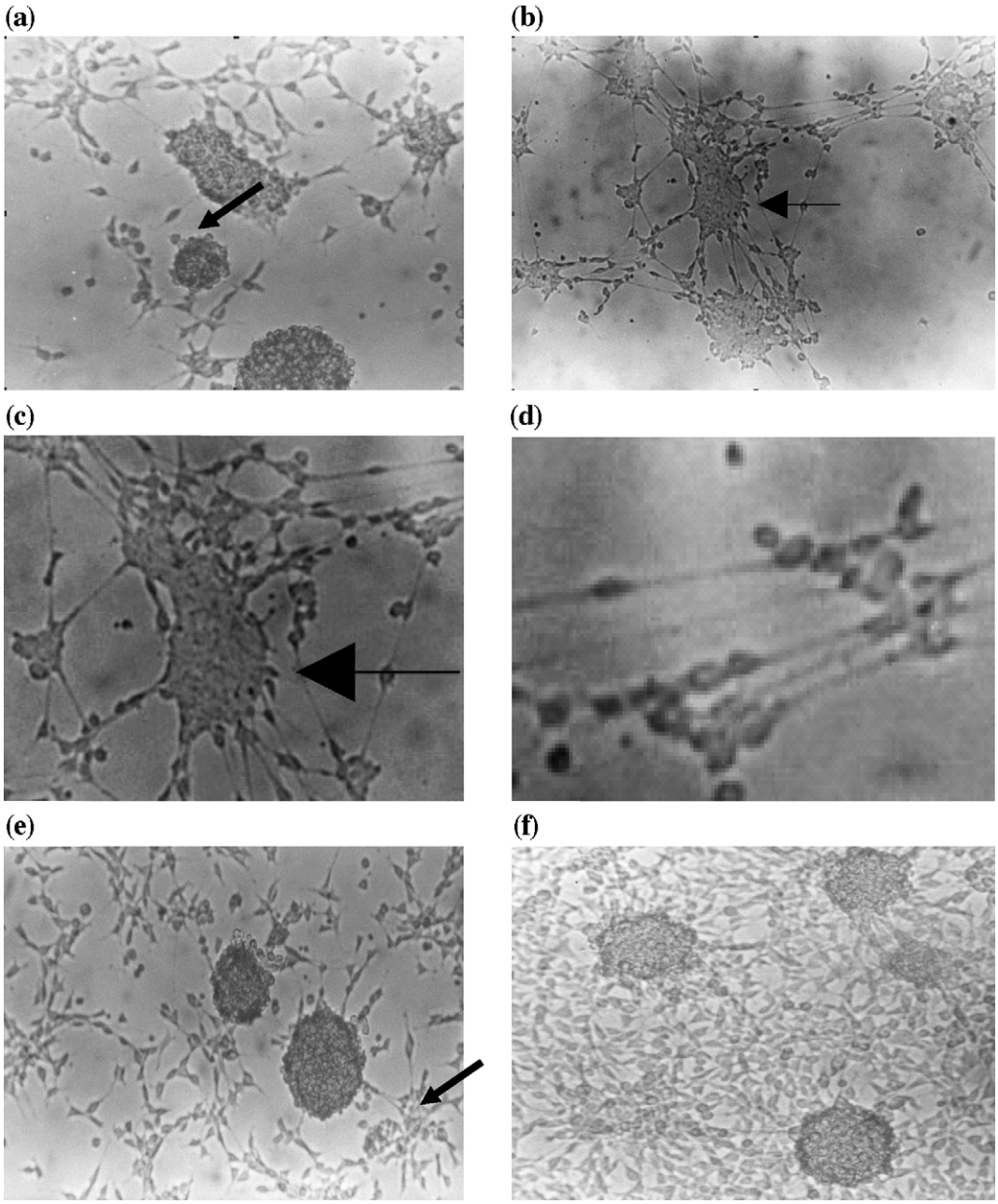



Fig. 3. Images obtained with an inverted phase contrast microscope of cells (300∗250 μm) cultivated on PEI polymeric films at different times of observation. (a) Cells begin to adhere to the substrate after 5 h in the medium culture. There are some accumulations of cells. These accumulations disappear after a few hours. Arrow shows a neuron. (b, c, d). Neuronal cells begin to interconnect themselves (8 h). Arrow shows neurons and their interconnection. (e) Cells continue to proliferate on the substrate (24 h). (f) Cells are confluent after 90 h. The whole FTO surface is covered by cells.

only a few percentage of cells even if they seem bigger than adhering cells because of their morphology (round and not extended as the neurons). Fig. 3b shows the adhesion after 8 h of culture. Cells begin to interconnect themselves since we could observe the development of fasciculated neurites. We observed Fig. 3c and d (which are magnification of Fig. 3b) that cells keep their neuronal characteristics (long prolongation with a soma).

Then, we studied the adhesion of cells onto the different substrates. In fact, we want to calculate the adhesion rate which is the number of cells plated on the surface 8 h after their seeding in the culture medium divided by the number of cells introduced in the culture medium (which is equal to $2.10^5$ cells/ml).

So the amounts of cells adhering on the four polymeric films are shown in Fig. 4 and Table 2. First, we can conclude that cells attach to all substrates. However, there are statistical differences in cell attachment among the substrates. Indeed, 51% of cells seeded onto PEI adhere and 46.5% on PPI but only 20.5% 19.5%, 18.5% adhere

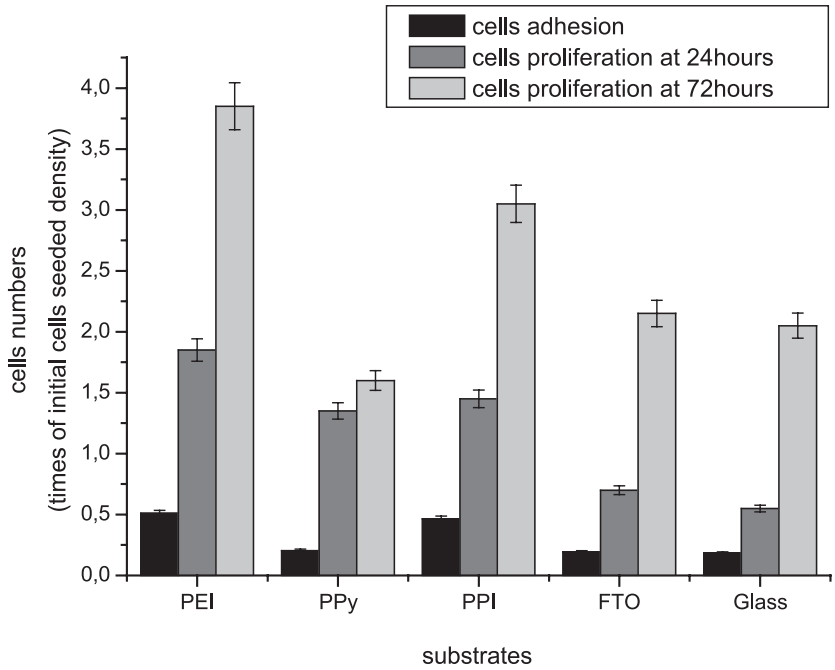



Fig. 4. Cell lines adhesion and proliferation on different substrates after 8, 24 and 72 h of culture. The numbers of cells were normalized to initial density of seeded cells (200,000 cells/ml) ($n = 3$ per substrates). The volume of the cell suspension used is 100 μl. All results are given at ± 5%.

on PPy, FTO uncovered and uncoated glass, respectively. So PEI and PPI allow more cells to adhere after seeding than other coatings. No quantitative study was done for PPPD since we observed that cells could not adhere on this polymeric film.

### 3.3.2. Cells proliferation

We studied the morphology of cells 24 and 90 h after they have been seeded.

Fig. 3e shows that cells proliferated well on the substrates after 24 h since their number was visibly increasing.

Fig. 3f shows that cells reached confluency after 90 h in the culture medium. The whole FTO surface is coated by cells and most of these cells are neuronal ones growing axons.

These photographs show that cells kept a normal morphology showing that the different substrates used are perfectly biocompatible.

Then, we studied proliferation of cells onto these different polymeric films 24 and 72 h after seeding (results are shown Fig. 4). Indeed, we calculated the proliferation rate, which is the number of cells on the surface 24 and 72 h after their seeding in the culture medium divided by the number of cells introduced in the culture medium.

Table 2
Adhesion rate and proliferation rate on the different substrates

| | Adhesion rate | Proliferation rate 24 h | Proliferation rate 72 h |
|---|---|---|---|
| PEI | 0.51 | 1.85 | 3.85 |
| PPI | 0.47 | 1.45 | 3.05 |
| PPy | 0.21 | 1.35 | 1.60 |
| FTO | 0.19 | 0.70 | 2.15 |
| Glass | 0.19 | 0.55 | 2.05 |

The increasing number of cells at the two time points demonstrates that the proliferation occurred on all substrates. As the initial cell densities were the same ($2.10^5$ cells/ml), at 24 h, cells were found to have more folds on expansion on PEI and PPI than on other substrates. So cells proliferate faster on these two latter polymeric substrates than on the other ones. At 72 h, cells continue to proliferate quickly on PEI and PPI and even on FTO and glass but not on PPy. Moreover, cells presented an abnormal morphology on PPy film.

## 4. Discussion

In this study, a rat neuronal cell line were able to adhere and proliferate on some of the polymeric films used. Phase contrast images showed that cell line plate and spread well on all the substrates tested but especially on PEI and PPI. There was no effect of the substrate on the cell morphology (see Fig. 3). These results correlated well with those of previous studies [43].

It was found that the number of cells adhering on FTO uncovered, glass and PPy is lower than the number of cells adhering on PEI or PPI. These results showed that cell lines are more likely to attach to polymeric films such as PEI or PPI. It is generally agreed that hydrophilicity of substrates affect biological responses, such as

cell adhesion and proliferation. Due to the presence of methyl group in PEI and PPI, these two polymers could be more hydrophobic than PPy. Another explanation for this difference of adhesion could be that amount and type of serum protein that adhered to substrates vary on different chemical surfaces, this can also affect cell behaviour on the substrates [44].

PPI and PEI have other interesting properties. Indeed, they are strongly bonded to the electrode surfaces during the electropolymerization step. Moreover, thanks to the presence of the amino groups onto the modified surfaces, it was possible to imagine that chemically modified electrodes can be used as substrates for cell culture. These films can grow thick enough because of their non-conducting properties, they give high impedance to the modified electrode providing a better protection to interferents and, they are insoluble in cold water and in main organic solvents. So these two polymers are very interesting for different applications in biology such as cell substrate but in pH sensors [45] or biosensors [46] and many other applications too.

## 5. Conclusion

In this present work, we show it is possible to make cells adhere onto polymeric films electrosynthesized by electrochemical oxidation. This method is cheap and easy and so could be a possible alternative to commonly used coating materials such as poly-L-lysine. All polymer substrates studied were able to allow neuronal cells of rats to adhere and proliferate. These cells are more likely to adhere on PEI and PPI than on PPy or PPPD. The information obtained should be correlated with other types of cells but is really encouraging for future experiments. Since we have already developed sensors [33,45] and microsystems [46] using polymer films coated on different surfaces, and since we showed that the culture of olfactory cells is possible on surfaces coated with PEI and PPI, we now want to develop a microsystem using these polymers as cell culture supports. This microsystem will consist in an olfactory neuron cultivated on polymeric films electrodeposited onto the silicon substrate. A neuron is surrounding with a medium culture. Different measurement systems can detect the activity of the neuron when we introduce a specific odour into the culture medium. Thus, as each olfactory cell is able to recognize a specific odour, the microsystem will be used as a sensor of odour, which can be very useful for water pollution control or drug's detection for instance.

## Acknowledgements

The authors want to thank Boris Lakard for his judicious advices concerning electrochemical experiments.

## References

[1] S. Velizarov, P. Raskmark, S. Kwee, The effects of radiofrequency fields on cell proliferation are non-thermal, Bioelectrochem. Bioenerg. 48 (1999) 177–180.
[2] P. Raskmark, S. Kwee, The minimizing effect of electromagnetic noise on the changes in cell proliferation caused by ELF magnetic fields, Bioelectrochem. Bioenerg. 40 (1996) 193–196.
[3] M.-P. Rols, C. Delteil, M. Golzio, J. Teissié, In vitro and ex vivo electrically mediated permeabilization and gene transfer in murine melanoma, Bioelectrochem. Bioenerg. 47 (1998) 129–134.
[4] G. Pucihar, L.M. Mir, D. Miklavi, The effect of pulse repetition frequency on the uptake into electropermeabilized cells in vitro with possible applications in electrochemotherapy, Bioelectrochemistry 57 (2002) 167–172.
[5] T. Kotnik, L.M. Mir, K. Flisar, M. Puc, D. Miklavi, Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses: Part I. Increased efficiency of permeabilization, Bioelectrochemistry 54 (2001) 83–90.
[6] R.M. Wightman, J.A. Jankowski, R.T. Kennedy, K.T. Kawagoe, T.J. Schroeder, D.J. Lesczyszyn, J.A. Near, E.J. Diliberto, O.H. Viveros, Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10754–10758.
[7] S.M. O'Connor, J. Andreadis, K.M. Shaffer, W. Ma, J.J. Pancrazio, D.A. Stenger, Immobilization of neural cells in three-dimensional matrices for biosensor applications, Biosens. Bioelectron. 14 (2000) 871–881.
[8] D. Kleinfeld, K.H. Kahler, P.E. Hockberger, Controlled outgrowth of dissociated neurons on patterned substrates, J. Neurosci. 8 (1998) 4098–4120.
[9] J.M. Corey, B.C. Wheeler, G.J. Brewer, Micrometer resolution silane-based patterning of hippocampal neurons: critical variables in photoresist and laser ablation processes fabrication, IEEE Trans. Biomed. Eng. 43 (1996) 944–955.
[10] P. Clark, P. Connolly, A.S.G. Curtis, J.A.T. Dow, C.D.W. Wilkinson, Topographical control of cell behavior: II. Multiple grooved substrata, Development 108 (1990) 635–644.
[11] P. Clark, Cell behaviour on micropatterned surfaces, Biosens. Bioelectron. 9 (1994) 657–661.
[12] D.V. Nicolau, T. Taguchi, H. Taniguchi, H. Tanigawa, S. Yoshikawa, Patterning neuronal and glia cells on light-assisted functionalised photoresists, Biosens. Bioelectron. 14 (1999) 317–325.
[13] M. Mrksich, C.S. Chen, Y. Xia, L.E. Dike, D.E. Ingber, G.M. Whitesides, Controlling cell attachment on contoured surfaces with self-assembled monolayers of alkanethiolates on gold, Proc. Natl. Acad. Sci. 93 (1994) 10775–10778.
[14] J.H. Lee, H.W. Jung, I. Kang, H.B. Lee, Cell behavior on polymer surfaces with different functional groups, Biomaterials 15 (1994) 705–711.
[15] T.G. van Kooten, J.M. Schakenraad, H.C. van der Mei, H.J. Busscher, Influence of substratum wettability on the strength of adhesion of human fibroblasts, Biomaterials 13 (1992) 897–904.
[16] A.S.G. Curtis, C. Wilkinson, B. Wojciak-Stotchard, Accelerating cell movement, Cell. Eng. 1 (1995) 35–38.
[17] R.M. Shelton, A.C. Rasmussen, J.E. Davies, Protein adsorption at the surface between charged polymer substrata and migrating osteoblasts, Biomaterials 9 (1988) 24–29.
[18] J.G. Steele, G. Johnson, P. Underwood, Role of serum vitronectin and fibronectin in adhesion of fibroblasts following seeding onto tissue culture polystyrene, J. Biomed. Mater. Res. 26 (1992) 861–884.
[19] D.A. Stenger, T.M. McKenna, Enabling Technologies for Cultured Neural Networks, Academic, London, 1994.
[20] K. Lewandowska, E. Pergament, C.N. Subenik, L.A. Culp, Cell-type specific adhesion mechanisms mediated by fibronectin adsorbed to chemically derivatized substrata, J. Biomed. Mater. Res. 26 (1992) 1343–1363.

[21] A.M. Mercurio, L.M. Shaw, Laminin binding proteins, BioEssays 13 (1991) 469–473.
[22] A. Dekker, T. Beugeling, H. Wind, A. Poot, A. Bantjes, J. Feijenand, W.G. van Aken, Deposition of cellular fibronectin and desorption of human serum albumin during adhesion and spreading of human endothelial cells on polymers, J. Mater. Sci., Mater. Med. 2 (1991) 227–233.
[23] T.G. Ruardij, M.H. Goedbloed, W.L.C. Rutten, Adhesion and patterning of cortical neurons on polyethylenimine- and fluorocarbon-coated surfaces, IEEE Trans. Biomed. Eng. 47 (2000) 1593–1599.
[24] M.E. Gomes, R.L. Reis, A.M. Cunha, C.A. Blitterswijk, J.D. de Bruijn, Cytocompatibility and response of osteoblastic-like cells to starch-based polymers: effects of several additives and processing conditions, Biomaterials 22 (2001) 1911–1917.
[25] A.P. Marques, R.L. Reis, Y.P. Bovell, A.M. Hunt, The biocompatibility of novel starch-based polymers and composites, Biomaterials 22 (2001) 1471–1478.
[26] Y. Bledi, A.J. Domb, M. Linial, Culturing neuronal cells on surfaces coated by a novel polyethyleneimine-based polymer, Brain Res. Protoc. 5 (2000) 282–289.
[27] E.M. Frödjdö, J. Westerlund, B. Isomaa, Culturing characterization of astrocytes isolated from juvenile rainbow trout (*Oncorhynchus mykiss*), Comp. Biochem. Physiol., Part A 133 (2002) 17–28.
[28] C. Boura, P. Menu, E. Payan, C. Picart, J.C. Voegel, S. Muller, J.F. Stolz, Endothelial cells grown on thin polyelectrolyte multilayered films: an evaluation of a new versatile surface modification, Biomaterials 24 (2003) 3521–3530.
[29] A. Blau, C. Weinl, J. Mack, S. Kienle, G. Jung, C. Ziegler, Promotion of neural cell adhesion by electrochemically generated and functionalized polymer films, J. Neurosci. Methods 112 (2001) 65–73.
[30] G. Herlem, C. Goux, B. Fahys, F. Dominati, A.M. Gonçalves, C. Mathieu, E. Sutter, A. Trokourey, J.F. Penneau, Surface modification of platinum and gold electrodes by anodic oxidation of pure ethylenediamine, J. Electroanal. Chem. 435 (1997) 259–265.
[31] G. Herlem, K. Reybier, A. Trokourey, B. Fahys, Electrochemical oxidation of ethylenediamine: new way to make polyethyleneimine-like coatings on metallic or semiconducting materials, J. Electroanal. Chem. 147 (2000) 597–601.
[32] M. Herlem, B. Fahys, G. Herlem, B. Lakard, K. Reybier, A. Trokourey, T. Diaco, S. Zairi, N. Jaffrezic-Renault, Surface modification of p-Si by a polyethylenimine coating: influence of the surface pre-treatment. Application to a potentiometric transducer as pH sensor, Electrochim. Acta 47 (2002) 2597–2602.
[33] B. Lakard, G. Herlem, M. Herlem, A. Etcheberry, J. Morvan, B. Fahys, Spectroscopic and ab initio study of polymeric films used as chemical sensors, Surf. Sci. 502–503 (2002) 296–303.
[34] I. Becerik, S. Suzer, F. Kardigan, Platinum–palladium loaded polypyrrole film electrodes for the electrooxidation of D-glucose in neutral media, J. Electrochem. Chem. 476 (1999) 171–176.
[35] E. Akalin, S. Akyüz, Theoretical study of IR spectra of paraphenylenediamine, Vibr. Spectrosc. 22 (2000) 3–10.
[36] J.C. Vidal, E. Garcia, J.R. Castillo, In situ preparation of overoxidized PPy/oPPD bilayer biosensors for the determination of glucose and cholesterol in serum, Sens. Actuators, B, Chem. 57 (1999) 219–226.
[37] M.D. Levi, M. Lapkowski, Mechanism of electron transfer during the course of oxidation of *N*-phenyl-*p*-phenylenediamine polythiophene and poly-3-methylthiophene coated electrodes: redox catalysis versus outer-sphere heterogeneous electron transfer, Electrochim. Acta 38 (1993) 271–279.
[38] X. Jing-Juan, C. Hong-Yuan, Amperometric glucose sensor based on coimmobilization of glucose oxidase and poly(*p*-phenylenediamine) at a platinum microdisk electrode, Anal. Biochem. 280 (2000) 221–226.
[39] V. Solis, T. Iwasita, M.C. Giordano, Paraphenylenediamine oxidation at a platinum electrode in acetonitrile solutions, J. Electroanal. Chem. 73 (1976) 91–104.
[40] B. Lakard, G. Herlem, B. Fahys, Ab initio study of the electrochemical polymerization mechanism of ω-diamines, J. Chem. Phys. 115 (2001) 7219–7226.
[41] B. Lakard, G. Herlem, B. Fahys, Ab initio study of the electrochemical polymerization mechanism leading from DETA to PEI, J. Mol. Struct., Theochem 593 (2002) 133–141.
[42] G. Herlem, B. Lakard, B. Fahys, Anodic oxidation of ω-amines for new polymer-modified electrodes, Recent Res. Dev. Electroanal. Chem. 3 (2001) 21–33.
[43] H.W. Ouyang, J.C.H. Goh, X.M. Mo, S.H. Teoh, E.H. Lee, Characterization of anterior cruciate ligament cells and bone marrow stromal cells on various biodegradable polymeric films, Mater. Sci. Eng., C, Biomim. Mater., Sens. Syst. 20 (2002) 63–69.
[44] Y. Tamada, Y. Ikata, Fibroblast growth on polymer surfaces and biosynthesis of collagen, J. Biomed. Mater. Res. 28 (1994) 783–788.
[45] G. Herlem, B. Lakard, M. Herlem, B. Fahys, pH-induced effect at electrode surfaces coated with linear polyethylenimine from electropolymerized ethylenediamine, J. Electrochem. Soc. 148 (2001) E435–E438.
[46] B. Lakard, G. Herlem, B. Fahys, M. de Labachelerie, W. Daniau, G. Martin, Chemical and biological sensors based on modified electrodes with electropolymerized diamines, Proceedings of the Fifth International Conference on Microreaction Technology, Springer-Verlag, Berlin, 2002, pp. 561–571.