Available online at www.sciencedirect.com
ScienceDirect
Bioelectrochemistry 72 (2008) 3–9
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Short communication

Synthesis and characterization of new electroactive polypyrrole–chondroitin sulphate A substrates

J. Serra Moreno [a], S. Panero [a,*], M. Artico [b], P. Filippini [c]

[a] Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[b] Department of Human Anatomy, Faculty of Pharmacy, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[c] Department of Technology and Health, Istituto Superiore Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Received 1 June 2007; received in revised form 31 August 2007; accepted 5 November 2007
Available online 13 November 2007

Abstract

Novel composite polypyrrole/chondroitin-4-sulphate films with cation-exchange properties were synthesized by the electrochemical polymerization of pyrrole in the presence of chondroitin-4-sulphate (CSA) sodium salt, acting as dopant anion at neutral pH. The negatively charged biomolecule was found to be permanently entrapped in the polypyrrole (PPy) membrane which resulted, as expected, facilitated in the mass transport by mobile cationic counterions. The porous nature of the substrates was identified as the most influential factor controlling the morphology. The morphology, in turn, affects the interaction between the material surface and the tissues on a cellular level. In this work in vitro analyses of human fibroblast response to polypyrrole/chondroitin-4-sulphate films were performed to focus on the different steps of cell reactions towards defined surface properties.

Keywords: Conducting polymer; Chondroitin-4-sulphate; Cell adhesion; Human fibroblast

1. Introduction

Tissue engineering develops specific tissues replacement based on synthetic scaffolds that guarantee tissue attachment and organization. One approach to create new scaffolds involves the design of materials that could enhance the cell attachment, proliferation and differentiation into functional tissues. In this regard, in vitro and in vivo experiments showed that biomaterials with electrical properties are able to stimulate bone cells and nerve regeneration [1,2]. Among different electroactive materials so far proposed as substrates, electroconducting polymers such as polypyrrole (PPy) or polyaniline (PANI) have been widely investigated. In particular, polypyrrole has been shown as suitable material for biomedical applications, since its biocompatibility with respect to mammalian cells has been proved [3]. Indeed, PPy-based devices have been developed as artificial muscles, surgical devices, drug delivery system, carrier of immobilized enzymes and biosensors [4,5]. The polymerization can be performed chemically or electrochemically by the oxidation of the pyrrole monomer in a proper electrolytic solution. In particular, the electrochemical polymerization allows to choose the synthesis parameters such as the nature and the concentration of the monomer and the supporting salt, as well as to control the charge density consumed during the electrochemical polymerization [6]. For instance, it has been shown that using a low synthesis current density the polymer chains are able to grow in an orderly way and the resulting structure reveals a smooth surface. On the other hand, high current density leads to irregular and porous surfaces. The electrochemical synthesis of polypyrrole, which can be performed in aqueous solution, allows to incorporate different bioactive anions such as proteins, DNA fragments or polyelectrolytes [7–9]. Such incorporation has attracted considerable interest as an avenue for tailoring the chemical and physical properties of the resulting polymer–polyelectrolyte composites and imparting appropriate activity, opening up new opportunities including genoelectronic devices, genetic analysis or probing of DNA charge transfer. The bioactive molecules, which can be grafted on the polymer chains, are expected to influence the response of living tissues, which they would come in contact to. In addition, a composite substrate that

* Corresponding author. Tel.: +39 0649913658; fax: +39 06491769.
E-mail address: stefania.panero@uniroma1.it (S. Panero).

1567-5394/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2007.11.002

combines biological activity with electrical properties represents an excellent substrate for the growth of cells. Panero et al. [10] reported the electrochemical synthesis of composite films comprising active polysaccharides (PSacch, namely hyaluronic acid HA and derivatives) and polypyrrole. The conductivity and morphology of the resulting PPy polymers strongly depended on the negative charge distributions in the HA macromolecules backbone. Other systems based on PPy and heparin (Hep) have been studied by Wallace et al. [11], who reported the electrochemical synthesis and biological characterization of these conducting films. The authors found that the PPy–Hep composites are well suited to support cells attachment and growth, since the films displayed high surface hydrophilicity and proper roughness. The polysaccharide molecules, so far studied to be incorporated into conducting films, are based on the repetitive units consisting of a hexosamina (glucosamina) and of another sugar (glucuronic acid), the sugar units along the chains being linked by α or β glycosidic bonds. The polyanionic chains of the polysaccharide molecules, having COO^- and SO_3^- groups, allow the PSacch macroions to interact with the positive charged PPy backbone during the electrosynthesis process. Although the incorporation of some polysaccharide compounds into PPy films has been already reported, the present work aims to complete the characterization extending the investigation to another member of the PSacch family, taking into consideration the chondroitin-4-sulphate A (CSA) molecule. Chondroitin sulphate, which is mainly covalently attached to proteins in the form of proteoglycan, is an ubiquitous component of all connective tissues of the extracellular matrix where it serves a number of functions. The biological functions are mainly due to the presence of rare oversulphated structural building units forming domain structures that interact specifically with other molecules, such as the regulation of neuronal patterning in the retina [12], interactions with fibronectin [13], neurite outgrowth promoting activity [14] and activation of plasminogen. CSA is also employed as an anti-inflammatory [15] and antirheumatic drug [16], where several controlled trials showed its effects with application in the therapy of osteoarthritis of the knee and articular cartilage with very good tolerability.

On the basis of these considerations, this paper describes the preparation and the characteristics of new polypyrrole–chondroitin-4-sulphate (PPy–CSA) films that can be used for tissue engineering applications. The in vitro interactions between PPy–CSA substrates and human fibroblast cells have been also tested. In particular the morphological modifications inducted by the cell-substrate interactions have been analyzed by SEM technique.

2. Materials and methods

2.1. Reagents

Pyrrole (>98%, Aldrich) was purified by passing it through a short column of neutral alumina (Aldrich) prior to use and kept refrigerated. Lithium perchlorate ($LiClO_4$ >99% Fluka) and chondroitin sulphate A sodium salt from bovine trachea (CSA, Sigma), were used without any prior purification. All synthesis solutions were performed in Milli-Q ultrapure water at room temperature.

2.2. Polymer synthesis

The PPy-based films were synthesized on indium tin oxide (ITO) substrates acting as working electrodes (surface area = 1.0 cm^2). The ITO electrodes were accurately washed with absolute ethanol before the synthesis. The pyrrole polymerization was performed at room temperature in the galvanostatic mode (current density ranging between 1.0 and 5.0 mA/cm^2) in a glass cell using a platinum counter electrode and a saturated calomel reference electrode SCE (electrode potential E = 0.241 V vs SHE). The electrosynthesis solutions were composed by pyrrole 1 mol/L and chondroitin-4-sulphate (2.0 or 5.0 mg/mL). In addition, some $PPyClO_4$ samples were also synthesized as control electrodes. The electrochemical polymerizations were performed using an AMEL potentiostat/galvanostat, model 7050, under computer control.

2.3. Electron microscopy

The morphology of the PPyCSA samples was studied by the scanning electron microscopy SEM technique (LEO model 1450VP).

2.4. Thermal properties

The thermal properties of the PPyCSA film and of its component CSA were investigated by Differential Scanning Calorimetry DSC, and by Thermal Gravimetric TGA analyses. DSC was carried out with a DSC 821 Mettler–Toledo at a scanning rate of 10 °C/min in the temperature range from −50 °C to 250 °C. PPyCSA films and CSA blank were previously quenched with N_2 before the DSC measurements.

TGA analyses were performed in a N_2 atmosphere using a TGA/SDTA 821 Mettler- thermobalance at a heating rate of 5 °C/min and in a temperature range of 25–300 °C.

2.5. Contact angle measurements

The advancing contact angle measurements were performed at room temperature by using the Wilhelmy plate method (Cahn Dynamic Contact Angle Analyzer DCA-312). The polypyrrole films were synthesized galvanostatically (current density I = 2.0 mA/cm^2, synthesis charge density 45 mC/cm^2) on a stainless steel foil (10 mm width × 15 mm height). The doping anions were CSA and perchlorate (2.0 mg/mL). Stainless steel foils were treated with HCl concentrated and washed with bi-distilled water before the electrosynthesis. The PPyCSA and $PPyClO_4$ samples were dried in a desiccator for 4 h at room temperature before performing the interfacial tension measurements. Then the dried samples were immersed in water (water plus for HPLC Carlo Erba, surface tension $\gamma_{(lv)}$ = 72.8 mN/m) at 200 μm/s immersion rate, in order to limit any possible swelling process.

2.6. Swelling measurements

The swelling characteristics of the PPyCSA samples were studied after their immersion in a phosphate buffer solution (PBS pH=7.4) at 37 °C for 4 days. The samples were galvanostatically synthesized at 3.0 mA/cm^2 (synthesis charge density 4.0 C/cm^2) from an electrolytic solution containing 1 mol/L pyrrole and 2.0 mg/mL CSA. After the synthesis, the PPy films were carefully dried overnight at 60 °C. The swelling degree (SD) was calculated according to the following formula:

$$\mathrm{SD}(\%) = \frac{W_{\mathrm{sw}} - W_{\mathrm{d}}}{W_{\mathrm{d}}} \quad (1)$$

where W_{sw} and W_{d} are the masses of the swollen and dried sample, respectively. After the immersion into the PBS solution, the samples were placed between two pieces of dry filter paper to wipe off the excess of water. The films were weighed until a constant weight, i.e., a constant mass, was reached. Each experiment was done in triplicate.

2.7. Electrochemical properties

The electrochemical characteristics of PPyCSA samples were studied by cyclic voltammetry (CV) tests using an EG&G Princeton Applied Research Mod. 263A potentiostat/galvanostat. The CV measurements were performed at the scan rate of 5 mV/s in a three-electrode glass cell where the PPy sample acted as working electrode, a platinum foil as the counter electrode and a SCE as the reference electrode. These experiments were conducted in aqueous 1 mol/L KCl solution previously deaerated by N_2 gas flow for 10 min. The stability of the PPyCSA samples after a sterilization process (UVA treatment at room temperature for 2 h in air) was evaluated by controlling the electroactivity of the sterilized electrodes during a voltammetric scan.

2.8. Cell culture

Lung diploid human fibroblasts WI-38 (#CCL-75) purchased from the American Type Culture Collection (USA) were maintained in RPMI medium (GIBCO-Invitrogen, MI, Italy) supplemented with 10% fetal calf serum, 1% non-essential amino acids, 100 units/mL penicillin and 100 μg/mL streptomycin. In order to sterilize ITO glasses, which are covered by PPy-based samples, they were put in six well plate and kept 2 h under UVA rays. After this time, the samples were washed twice in fresh medium and seeded with fibroblasts at the concentration of 2×10^5 cell/mL. Plates were maintained at 37 °C in humidified atmosphere for at least 7 days.

2.9. Cell examination by scanning electron microscopy

After 7 days of growth, the cells were fixed with 2.5% glutaraldehyde for 20 min at room temperature and washed twice in 0.1 M cacodylate buffer. All samples were postfixed in 1% osmium tetroxide for 30 min at room temperature, dehydrated through graded ethanol solutions, critical point, dried in $CO_{2(g)}$ and gold coated by sputtering. The samples were examined with a Cambridge 360 stereoscan electron microscope.

3. Results and discussion

3.1. Synthesis and morphology characterization

In our previous work [10], we demonstrated that it is possible to immobilize the bioactive polysaccharide molecules into the PPy matrix due to the large distribution of negatively charge groups in the PSacch backbones. It was also shown that the synthesis parameters strongly affected the electrochemical and morphological characteristics of the PPy-based samples. In this work we have extended the study to another polysaccharide compound, namely the chondroitin sulphate A molecule, whose chain-shaped structure exposes sulphonic and carboxylic groups on the surface.

Many authors reported the influence of the current regimes on the morphology of PPy-based substrates, showing that the electropolymerization conducted at high current density resulted in rough and porous surface characterized by large aggregates (cauliflower morphology) [17]. In this study we confirm the results so far presented considering the PPy-X (X=ClO_4^-, DS^-, ToS^-) samples formation. In addition, we show that the case of large polyanions as doping agents, such as CSA, strongly affect the surface properties of the PPy-based films. In particular, the affinity of the macroanion to water may influence the interactions of the polysaccharide chains and the pyrrole units during the electrosynthesis and this, in turn, may affect the local organization of the resulting PPyCSA substrate [18]. Fig. 1 shows the morphological characteristics of the PPyCSA films synthesized at 1.0 mA/cm^2 from electrosynthesis solutions containing CSA (2.0 or 5.0 mg/mL). The SEM images show that the surface properties of the PPy films are determined by the macroanion concentration. In fact, samples electropolymerized with lower polysaccharide concentration reveal smooth morphology since the CSA-CSA interactions decrease during the synthesis (Fig. 1a). Higher CSA concentration results in irregular PPy surface (Fig. 1b).

3.2. Thermal properties

Fig. 2 compares the DSC response of the pure CSA powder and of the PPyCSA film. As expected, the thermal property of the chondroitin sulphate A resembles the behavior of the polysaccharide compounds, showing two endothermic peaks (at −9.52 °C and around 177.32 °C) related to the water molecules bonded to the polymer chains, and an exothermic peak at 233.86 °C, due to the irreversible polysaccharide degradation process, clearly revealed by the subsequent DSC cooling scan. Both effects, low-temperature phase transition and dehydration, can be related to intramolecular and intermolecular hydrogen bonding capabilities and to hydration and cross-linking effects [19].

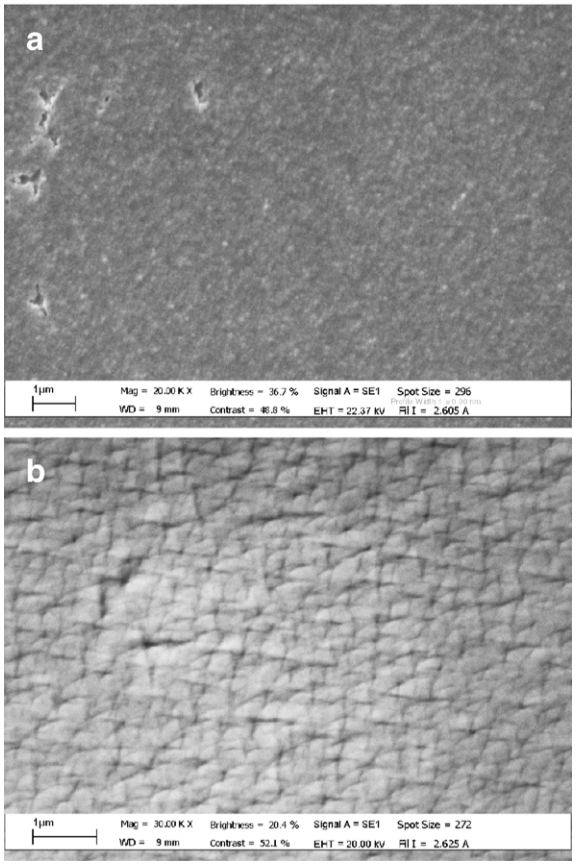

Fig. 1. Scanning electron micrographs of PPyCSA films synthesized at the current density $I = 1.0$ mA/cm^2 from synthesis solutions containing CSA 2.0 mg/mL (a) or 5.0 mg/mL (b).

The thermal scan of the PPyCSA sample shows two endothermic peaks at −4.37 °C and 10 °C due to the water bonded to the CSA molecules (see before) and to the water adsorbed during the synthesis process. The subsequent dehydration process points out an endothermic peak at 134.5 °C. Moreover, no thermal degradation phenomena appear at higher temperature, this confirming the ability of the polypyrrole ordering matrix to decrease the dehydration temperature of the polysaccharide and also to limit the CSA degradation processes.

Fig. 3 shows the thermogravimetric response of the PPyCSA film and of its component CSA. As expected by the high water content in the samples, the weight loss curves show a gradual decrease up to 200 °C due to the water release and a subsequent thermal process which starts at 227 °C. From the comparison of the TGA curves here reported, we confirm that the PPy-based films are able to assure high stability and limited weight loss even at elevated temperature.

3.3. Contact angle measurements

The surface properties of a biomaterial are of utmost importance for its biocompatibility. For instance, the cell-material interaction is strongly influenced not only by the surface topography but also by the surface chemistry including surface charge and wettability [20,21]. In this work the wettability of PPyCSA and PPyClO$_4$ samples was studied by contact angle measurements. The results reveal that the presence of the CSA in the polymer substrate assures to the film a high degree of hydrophilicity ($\theta = 53°$) while the ClO$_4$ control anion gives poor wettability ($\theta = 104.26°$). These surface characteristics are related to the chemical properties of the CSA polysaccharide anion, which shows different polar groups along the chains, such as hydroxyl, carboxylic and sulphate groups. The experimental results confirm the high water binding ability of CSA, which is an important property for applications in the field of orthopaedic implantation and tissue engineering.

3.4. Swelling properties

The water uptake experiments were carried out following the soaking properties of some PPyCSA films after their immersion in a buffered solution. After 4 days, the polymeric films appeared still compact and homogeneous and they reported a swelling degree of about 19%.

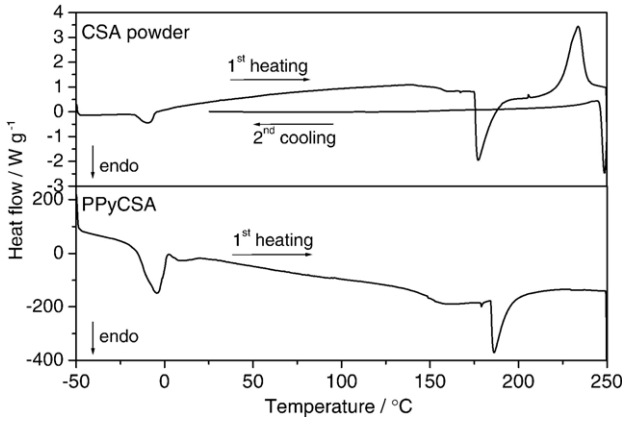

Fig. 2. Differential Scanning Calorimetry scans of CSA blank powder and PPyCSA film. Thermal scan rate 10 °C/min; $N_{2(g)}$ (60 mL/min) flux.

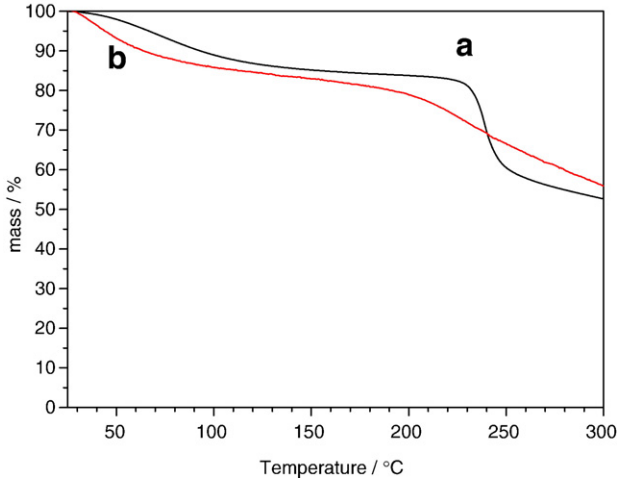

Fig. 3. Thermogravimetric curves of CSA blank powder (curve a) and PPyCSA film (curve b) at 5 °C/min in $N_{2(g)}$ (60 mL/min) gas flow.

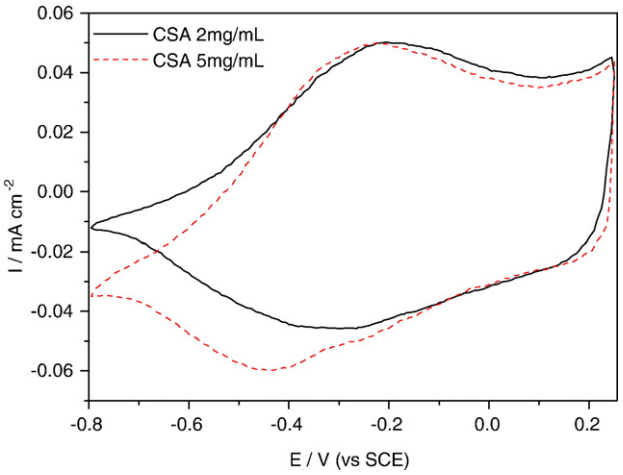

Fig. 4. Cyclic voltammetry of the PPyCSA films synthesized in different CSA solutions: 2.0 mg/mL (−) and 5.0 mg/mL (—). Samples synthesized at 1.0 mA/cm^2. Scan rate 5 mV/s.

3.5. Electrochemical properties

The response of biological tissue to polymer materials in vivo is one of the most fundamental and complex challenges of modern restorative medicine. To promote tissue formation and integration, a biocompatible material provides a surface which enables cell adhesion, spreading, proliferation and migration. Moreover, electric fields applied to damaged tissues help control cell identity, position and movement which are relevant to any biomedical phenomenon development [22].

In this work, the electrochemical properties of the PPyCSA samples have been analyzed studying the effect of the synthesis anions concentration (2.0 or 5.0 mg/mL) on the electrochemical characteristics of the resulting films. Fig. 4 reports in comparison the CV response of two PPyCSA samples synthesized in different CSA solutions. The PPyCSA samples show well defined voltammetric profiles though the CSA (2.0 mg/mL)-based film reveals a better electrochemical response in terms of reversibility and doping level (Y=22.50%) in comparison to Y=21.04% for CSA(5.0 mg/mL)-based sample. The results can be explained considering the different CSA-water interactions and anion mobility in the two synthesis solutions thus influencing the overall electrosynthesis process.

Finally, the stability of a PPyCSA electrode after a sterilization process was studied. Fig. 5 reports in comparison the voltammetric response of the sample before and after the UVA treatment. The PPyCSA electrode shows its excellent electrochemical characteristics even after the sterilization process.

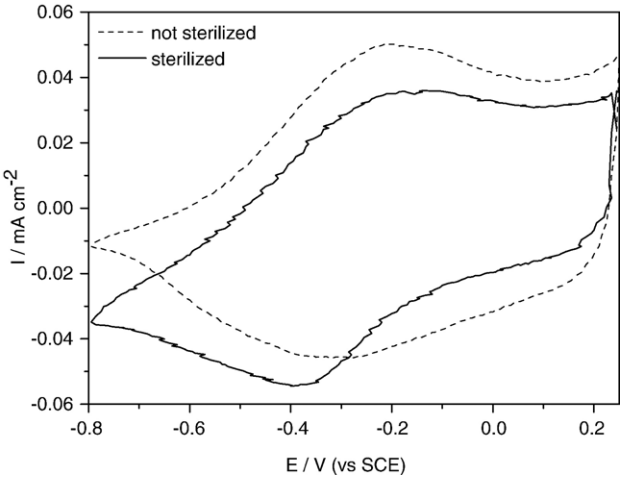

Fig. 5. Cyclic voltammogram of PPyCSA film samples before and after UVA sterilization. Scan rate of 5 mV/s.

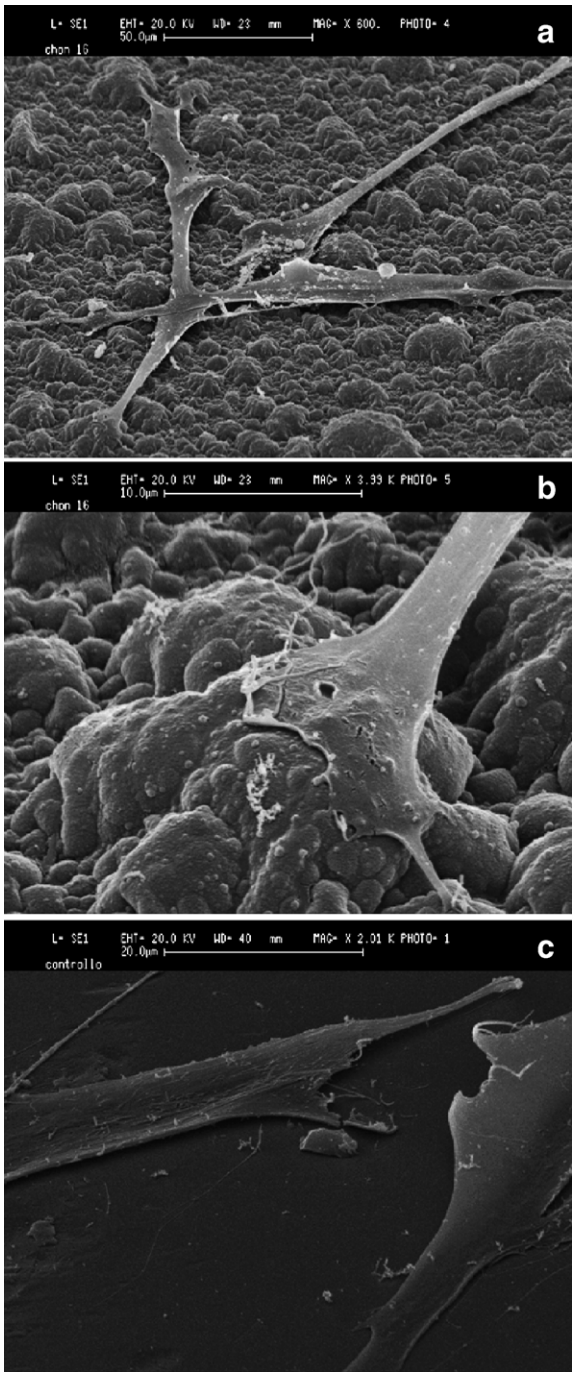

Fig. 6. Scanning electron micrographs of human fibroblast cells seeded onto PPyCSA: at 600× (a) and at 3.99 kX (b). ITO control sample at 2.01 kX (c).

3.6. Cell activity on PPyCSA substrates

In this work we confirm that the cellular response is strongly influenced by the polymeric substrate morphology. For instance, the PPyCSA samples synthesized at the high current density (I=5.0 mA/cm^2) in a 5.0 mg/mL CSA synthesis solution show a rough surface morphology. On this surface, the human fibroblasts present an elongated morphology with few points of adhesion. The ITO control sample indeed presents a total cells adhesion on the substrate (Fig. 6a, b and c).

By choosing the proper electrosynthesis conditions, i.e. lower current synthesis of I=1.0 mA/cm^2 and in 2.0 mg/mL CSA synthesis solution, the resulting PPyCSA samples look like smooth and homogeneous films that offer to the cells a suitable surface for adhesion and spreading (Fig. 7a) [23]. Similar results are also observed for PPyCSA samples pre-treated with FCS serum for 2 h before cell tests (Fig. 7b). Even in this case the human fibroblast cells have similar morphology than those totally adhered on the ITO control sample.

It is well known that culture cells adhesion on different surfaces is due to the presence of serum proteins that works as "bridges" between adhesion molecules, expressed on cell membrane, and the biomaterial [24]. In our study we have observed that human fibroblasts interact with PPyCSA films even with those not-pretreated with FCS. These data indicate that the PPyCSA substrates offer a proper surface for cell adhesion and growth due to the presence of the CSA biomolecule, which is able to interact with cell proteins through its RGD sequence [25,26], and probably the CSA exposed on these films play a pivotal role during these processes. This is underlined also by the evidence that the pretreatment with serum proteins does not improve the surface characteristics.

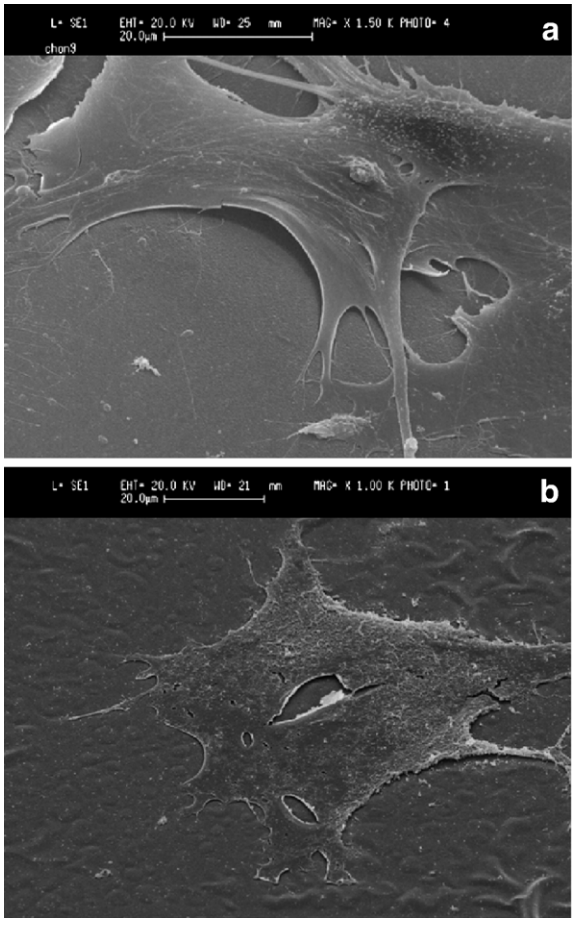

Fig. 7. Scanning electron micrographs of human fibroblast cells onto PPyCSA substrates not-treated at 1.50 kX (a) and treated with FCS at 1.00 kX (b).

4. Conclusions

In this work, the electrosynthesis of polypyrrole–chondroitin sulphate A films has been presented. We have reported the high influence of the synthesis parameters, such as current density and doping anion concentration, in the chemical and physico-chemical properties of the resulting samples. The polypyrrole samples synthesized at 1.0 mA/cm^2 from 2.0 mg/mL CSA starting solution have shown a better electrochemical performance and morphology. The PPyCSA substrates have demonstrated fast kinetics characteristics and good stability even after a sterilization process. The morphology, in turn, is strongly related to the current density regime and CSA concentration. These two factors, surface chemistry and morphology, influence the cell-substrate interactions, as the in vitro experiments clearly confirm. Indeed, the SEM analyses have shown a good cell adhesion on the smooth PPyCSA films even without any FCS supplement.

Acknowledgments

This work was carried out with the financial support from the Sapienza University of Rome (Ateneo funds) and the Italian Ministry of Research and University, MIUR (PRIN 2005).

References

[1] C.E. Schmidt, V.R. Shastri, J.P. Vacanti, R. Langer, Stimulation of neurite outgrowth using an electrically conducting polymer, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8948–8953.
[2] A. Kotwal, C.E. Schmidt, Electrical stimulation alters protein adsorption and nerve cell interactions with electrically conducting biomaterials, Biomaterials 22 (2001) 1055–1064.
[3] J.H. Collier, J.P. Camp, T.W. Hudson, C.E. Schmidt, Synthesis and characterization of polypyrrole–hyaluronic acid composite biomaterials for tissue engineering applications, J. Biomed. Mater. Res. A 50 (2000) 574–584.
[4] A. Guiseppi-Elie, G.G. Wallace, T. Matsue, Chemical and biological sensors based on electrically conducting polymers, in: T.A. Skotheim, R.L. Elsenbaumer, J.E. Reynolds (Eds.), Handbook of Conducting Polymers, Marcel Dekker, New York, 1998, pp. 963–991.
[5] X.Y. Cui, J.F. Hetke, J.A. Wiler, D.J. Anderson, D.C. Martin, Electrochemical deposition and characterization of conducting polymer polypyrrole/PSS on multichannel neural probes, Sens. Actuators, A, Phys. 93 (2001) 8–18.
[6] T.F. Otero, E. Angulo, Comparative kinetic studies of polypyrrole electrogeneration from acetonitrile solutions, J. Appl. Electrochem. 22 (1992) 369–375.
[7] J. Rick, T.C. Chou, Using protein templates to direct the formation of thin-film polymer surfaces, Biosens. Bioelectron. 4 (2006) 544–549.
[8] J. Wang, M. Jiang, Towards genolelectronics: nucleic acid doped conducting polymers, Langmuir 16 (2000) 2269–2274.

[9] T.F. Otero, J.M. Sansiñena, Influence of synthesis conditions on polypyrrole–poly(styrenesulphonate) composite electroactivity, J. Electroanal. Chem. 412 (1996) 109–116.
[10] S. Panero, G. Abbati, D. Renier, V. Crescenzi, Electrically conductive polymeric biomaterials the process for their preparation and the use thereof in the biomedical and health-care, PCT Int. Appl. WO 2003000309 (2003).
[11] B. Garner, A.J. Hodgson, G.G. Wallace, P.A. Underwood, Human endothelial cell attachment to and growth on polypyrrole–heparin is vitronectin dependent, J. Mater. Sci., Mater. Med. 10 (1999) 19–27.
[12] P.A. Brittis, D.R. Canning, J. Silver, Chondroitin sulfate as a regulator of neuronal patterning in the retina, Science 255 (1992) 733–736.
[13] F.J. Barkalow, J.E. Schwarzbauer, Interactions between fibronectin and chondroitin sulfate are modulated by molecular context, J. Biol. Chem. 269 (1994) 3957–3962.
[14] S. Nadanaka, A. Clement, K. Masayama, A. Faissner, K. Sugahara, Characteristic hexasaccharide sequences in octasaccharides derived from shark cartilage chondroitin sulfate D with a neurite outgrowth promoting activity, J. Biol. Chem. 273 (1998) 3296–3307.
[15] F. Ronca, L. Palmieri, P. Panicucci, G. Ronca, Anti-inflammatory activity of chondroitin sulfate, Osteoarthr. Cartil. A 6 (1998) 14–21.
[16] D. Uebelhart, E.J. Thonar, P.D. Delmas, A. Chantraine, E. Mignon, Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study, Osteoarthr. Cartil. A 1 (1998) 39–46.
[17] H. Naarmann, D. Schmeiber, A. Bartl, L. Dunsch, W. Göpel, Electronic and magnetic properties of polypyrrole films depending on their one-dimensional and two-dimensional microstructures, Synth. Met. 93 (1998) 43–58.
[18] D. Zhou, C.O. Too, G.G. Wallace, Synthesis and characterisation of polypyrrole/heparin composites, Reac. Func. Pol. 39 (1999) 19–26.
[19] A.J. De Andrés Santos, A. Velasco Martín, E. Hernández Velasco, J. Martín Gil, F.J. Martín Gil, Thermal behaviour of aqueous solutions of sodium hyaluronate from different commercial sources, Thermochim. Acta 242 (1994) 153–160.
[20] M.R. Kreke, A.S. Badani, J.B. Brady, R.M. Akers, A.S. Goldstein, Modulation of protein adsorption and cell adhesion by poly(allylamine hydrochloride) heparin films, Biomaterials 26 (2005) 2975–2981.
[21] 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.
[22] R.F. Valentini, T.G. Vargo, J.A. Gardella, P. Aebischer, Electrically charged polymeric substrates enhance nerve fibre outgrowth in vitro, Biomaterials 13 (1992) 183–190.
[23] M. Tirrell, E. Kokkoli, M. Biessalski, The role of surface science in bioengineered materials, Surf. Sci. 500 (2002) 61–83.
[24] M.T. Santini, G. Rainaldi, P.L. Indovina, Apoptosis, cell-adhesion and the extracellular matrix in the three-dimensional growth of multicellular tumor spheroids, Crit. Rev. Oncol./Hematol. 36 (2000) 75–87.
[25] J.H. Lee, H.W. Jung, I.K. Kang, H.B. Lee, Cell behaviour on polymer surfaces with different functional groups, Biomaterials 15 (1994) 705–711.
[26] E. Ruoslahti, RGD and other recognition sequences for integrins, Annu. Rev. Cell Dev. Biol. 12 (1996) 697–715.