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Dexamethasone electrically controlled release from polypyrrole-coated nanostructured electrodes

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Abstract One of the key challenges to engineering neural interfaces is to reduce their immune response toward implanted electrodes. One potential approach to minimize or eliminate this undesired early inflammatory tissue reaction and to maintain signal transmission quality over time is the delivery of anti-inflammatory biomolecules in the vicinity of the implant. Here, we report on a facile and reproducible method for the fabrication of high surface area nanostructured electrodes coated with an electroactive polymer, polypyrrole (PPy) that can be used to precisely release drug by applying an electrical stimuli. The method consists of the electropolymerization of PPy incorporated with drug, dexamethasone (DEX), onto a brush of metallic nanopillars, obtained by electrodeposition of the metal within the nanopores of gold-coated polycarbonate template. The study of the release of DEX triggered by electrochemical stimuli indicates that the system is a true electrically controlled release system. Moreover, it appears that the presence of metallic nanowires onto the electrode surface improves the adherence between the polymer and the electrode and increases the electroactivity of the PPy coating.

1 Introduction

Improving the interfacial contact between electrodes and biological tissues is one of the main challenge that must be faced for the development of long-term performance active implantable devices, such as neural prostheses used to restore damaged neurological function by electrical stimulation of nerves in the peripheral and central nervous systems. The development of such devices is, indeed, currently limited by the drop of their electrical performances over extended periods, mainly due to an undesired inflammatory tissue reaction following the surgically implantation of the neural stimulator [1, 2]. This leads to the formation of a glial scar between the electrode and the nerve, acting as a resistance to signal transmission. One of the biomaterials strategies currently explored to minimize or eliminate this undesired early inflammatory tissue reaction and to maintain signal transmission quality over time is the delivery of anti-inflammatory biomolecules in the vicinity of the implant. One of the drugs commonly used for its intense anti-inflammatory activity is the dexamethasone (DEX), a synthetic glucocorticoid hormone. Systemic injections of DEX were shown to reduce tissue reaction around the implant in the central nervous system [3, 4], but this kind of DEX administration is very expensive and can cause serious side effects, such as myopathy and diabetes [5, 6]. To overcome these problems, local delivery of drugs by the implant itself is a promising and more effective strategy. In this respect, Zhong et al. [7] developed a DEX eluting nitrocellulose-based coating on silicon neural probes that was proven to attenuate the inflammatory response and to reduce neuronal loss in the vicinity of the coated probes. However, this passive delivery system, based on simple drug diffusion, does not allow any control of the released drug doses. Therefore, there is currently an increased interest in developing electrically controlled drug delivery systems using electroactive conducting polymers as coatings. Indeed, due to their unique redox properties that allow

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controlled ionic transport through the polymeric film, these polymers have been considered for drug delivery applications. Among conjugated polymers, polypyrrole (PPy) is particularly attractive because of its easy and efficient electrosynthesis from aqueous solutions, biocompatibility, good environmental stability and low voltage actuation [8]. Up to now, PPy has already been studied for controlled delivery of different biomolecules, such as adenosine 5′-triphosphate (ATP) [9], neurotrophin [10, 11] and dexamethasone [12]. In the latest, Wadhwa et al. reported on a PPy-coated electrode which allows a local and electrical control over the amount of DEX released. However, several problems were highlighted, in particular chemical and mechanical instability of the polymer. They observed macroscopic cracks on the polymer surface after a few electrochemical stimulation cycles, suggesting that the film undergoes physical stresses upon swelling and shrinking cycles, leading to its delamination and breakdown. This is, of course, dedrimental for the long-term performances.

In the present work, we report on a successful and reproducible process to elaborate PPy nanostructured electrodes for improved electrically controlled and local delivery of anti-inflammatory [13]. The strategy developed for preparing the nanobioelectrodes is based on the hard templating method that presents the significant advantage of leading to the production of nanotubes or nanowires with monodisperse and controlled diameters and lengths [14]. In this study, platinum was electrodeposited into the nanopores of an Au-coated polycarbonate membrane. The further dissolution of the membrane leads to the formation of a brush of vertically aligned metallic nanowires on the substrate. Then, a one step electropolymerization procedure was used to deposit PPy films doped with DEX onto the Pt nanopillar brush. By controlling the polymerization time, nanostructured electrodes including PPy/Dex films of different thicknesses were prepared. Electrochemically controlled DEX release was achieved and it was found that the system is a true electrically controlled release system. A study of the electroactivity and morphology of the nanostructured PPy/DEX electrodes before and after drug release was carried out by cyclic voltammetry and scanning electron microscopy (SEM), respectively. It was found that the presence of metallic nanopillars onto the electrode surface improves the adherence between the polymer and the electrode and increases the electroactivity of the PPy coating.

2 Materials and methods

2.1 Preparation of Pt nanopillar brushes

The nanopillar brushes were prepared by an electrochemical template method. The electrochemical growth of Pt nanowires was carried out in a commercial one compartment Teflon cell [CH Instruments] at room temperature with a Pt disk counter-electrode and an Ag/AgCl reference electrode. The electrosynthesis was controlled using an EG&G Princeton Applied Research 273A potentiostat/galvanostat. Nanoporous polycarbonate (PC) track-etched membranes with a thickness of 21 μm, pore diameter of 150 nm and a pore density of 10^8 cm^{-2} were supplied by *it4ip* [15] and used as templates. A 450 nm gold layer was previously evaporated on one side of the membrane, serving as working electrode. The Pt nanowires were electrodeposited potentiostatically on disk samples (Area = 0.282 cm^2) at 0 V from a homemade solution of 0.01 M $Na_2PtCl_6{\cdot}6H_2O$ and 0.5 M H_2SO_4 in de-ionized water. The amount of material deposited within the nanopores was controlled by the total electrical charge passed during the electrodeposition process. After deposition of the Pt nanowires, the PC template was removed by dissolution in dichloromethane. The brush of Pt nanopillars attached to the gold surface was finally rinsed with a 0.5 M NaOH solution in order to remove any trace of PC onto the nanowires.

2.2 Polypyrrole film synthesis

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

2.3 In vitro dexamethasone drug release study

Electrical stimulation of PPy/DEX nanopillar brushes was performed by cyclic voltammetry (CV) using an EG&G Princeton Applied Research 273A potentiostat. For drug release studies, the electrolyte was a 20 mM PBS with 150 mM NaCl (pH 7, room temperature). The voltage was switched from −0.8 to 0.9 V at a scan rate of 100 mV/s. The amount of released DEX was quantified by UV absorbance at 242 nm (a characteristic band of DEX) using a *Cary 500 scan UV–vis–NIR*. The readings were taken after every five cycles until 50 cycles, then after every 10 cycles until 100 cycles and finally after 150 cycles. To ensure that the release is mainly caused by the electrical stimulus, a control was set up in which each sample was

simply immersed in PBS solution (without any cyclic potential stimulus) and UV measurements were done after 10, 20 and 30 min. These results were compared with the first sets of CV cycles (considering that 1 CV cycle $\sim$1 min at 100 mV/s).

2.4 Scanning electron microscopy (SEM)

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

3 Results and discussion

3.1 Preparation and structural characterization of nanostructured polypyrrole-coated electrodes

The efficiency of the developed process, based on the hard templating method, for preparing nanostructured electrodes was checked by analyzing the electrode surfaces at each step of the preparation using SEM. First, a nanopillar brush was synthesized by electrodeposition of platinum within the nanopores of a PC membrane. Figure 1 shows a typical picture of a brush of self-standing Pt nanopillars obtained after dissolution of the PC template. As expected, the diameters of the nanopillars are quite monodisperse and correspond to the pore diameter of the PC membrane used as template $\sim$150 nm. The mean pillar height (h_p) is controlled by the electrical charge passing through the system during the synthesis. The potential was automatically turned off when the desired charge was reached. For the templates used in this study, PC membranes with pore diameter (Φ_p) = 150 nm and pore density (d) = 10^8/cm^2, the mean pillar height can be related to the electrical charge by the following empirical relation: 15.5 mC/cm^2 for an h_p = 500 nm. The growing on the electrode substrate of a Pt nanopillar brush with such structural characteristics (d = 10^8/cm^2, Φ_p = 150 nm, h_p = 500 nm) leads to an increase of $\sim$25% of the active electrode surface. One serious advantage of our proposed method for preparing nanostructured electrodes, compared to the recently reported method based on the use of self-assembly of polystyrene nano-beads as template [16], is that here the increase of active surface area can be adjusted by playing on the different structural parameters of the brush. Indeed, the pore diameter of the used template can be fixed between 30 and 200 nm and the pore density between 10^6 and 5 $\times$ 10^9/cm^2. The height of the nanopillars can be tuned between only a few nanometers to a few microns. Though, to avoid the breaking of the Pt nanopillars when removing the PC template, their aspect ratio (height/diameter) must be kept below 4.

In a second step, PPy/DEX films of various thicknesses were synthesized on the top of Pt nanopillar brushes by a one step electropolymerization at 0.8 V vs Ag/AgCl. As the growing PPy backbone chain is positively charged, the DEX ions present in the electropolymerization solution are incorporated during electrosynthesis in order to maintain the electroneutrality within the film. Figure 2 shows a typical image of the surface a PPy/DEX film synthesized on a nanostructured electrode (Q_{PPy} = 55 mC/cm^2). The thickness of the polymeric film can be roughly estimated from the electrical charge consumed during the electrosynthesis.

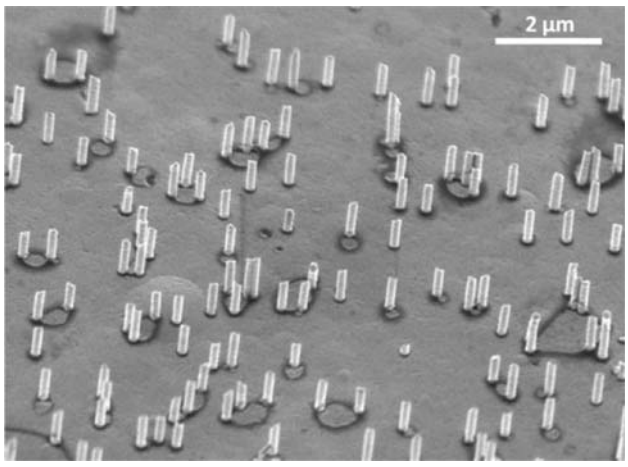


Fig. 1 SEM picture of a brush of self-standing Pt nanopillars on a gold surface (Q_{Pt} = 24 mC/cm^2, h_p = 800 nm, Φ_p = 150 nm, d = 10^8/cm^2)

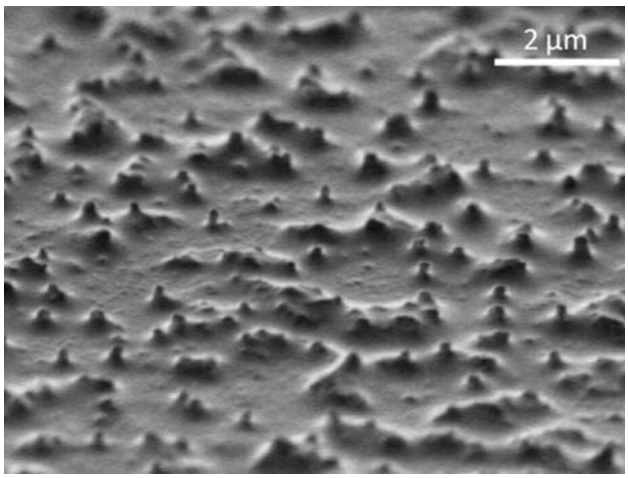


Fig. 2 SEM picture of a PPy/DEX film on a Pt nanopillar brush (Q_{PPy} = 54.7 mC/cm^2)

3.2 In vitro dexamethasone drug release study

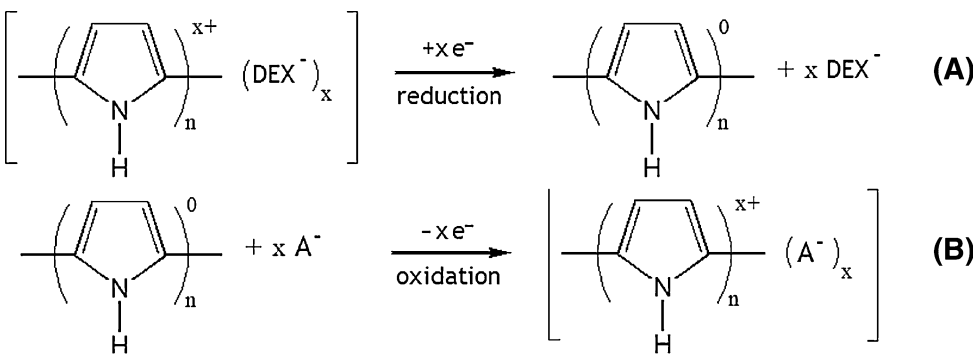
The influence of the well-defined nanostructures established on the electrode surface on their release performances were then evaluated. Drug release from PPy/DEX films is based on the particular feature of PPy that can undergo redox cycling between conductive and insulating states. This redox switching is accompanied by swelling and shrinking of the polymer material due to the incorporation or release of hydrated ions A^- (Scheme 1). In this work, A^- is the anionic DEX that was released from PPy/DEX films by switching the potential between −0.9 and 0.8 V using cyclic voltammetry at 100 mV/s.

Cyclic voltammetry is an electroanalytical technique that allows assessing the reduction and oxidation peaks of the polymer. Figure 3 present cyclic voltammograms (measured current against the applied voltage at the working electrode) recorded on a nanostructured PPy/DEX electrode after different number of scans. On Fig. 3a, the switching properties of PPy can be clearly distinguished: two peaks appear at −0.2 and +0.15 V, corresponding to the reduction and oxidation of the nanostructured PPy/DEX film (Scheme 1), respectively. It should be pointed out that the redox peaks of PPy/DEX films on nanostructured electrodes appear at significantly lower different potentials than on planar metallic electrodes, where, as reported by Wadhwa et al. [12], the reduction and oxidation peaks are located at −0.34 and +0.49 V, respectively. These differences can be attributed to the influence of the Pt nanopillars on the PPy film electrosynthesis process and, consequently on its resulting morphology and electrical properties. These significant shifts of the redox potentials represent a serious advantage for the long-term performances of the electrodes, as lower applied potentials or currents could be used for the release of drug.

The release of DEX ions is however not an instantaneous process. To be detected, the anions have to diffuse from the inner part of the film to the surface and then, to the buffer solution. The diffusion time of these bulky ions will depend on the PPy film density. Moreover, if the CV scan rate applied for the release is too high, DEX ions will remain in the PPy film during the potential switching and can therefore bind again to the positively charged polymer backbone upon oxidation, preventing the release of DEX in the solution. A too low CV scan rate could also bring some problems, as if the negative potential is applied for a too long time, the film becomes electrically insulating and, the recovery of the conductivity upon re-oxidation becomes less easier. It appears that cyclic voltammetry stimulation at a scan rate of 100 mV/s is a good compromise between effective release properties of drug and preservation of the electrical characteristics of the film. At each CV cycle, a small amount of the total DEX ions contained in the PPy film is released. The quantification of the DEX liberated was done using UV absorbance at 242 nm. The cumulative DEX release profiles over 150 CV for two different samples: a *thin* PPy/DEX film ($Q_{PPy} = 27.4$ mC/cm^2) and a *thick* PPy/DEX film ($Q_{PPy} = 700$ mC/cm^2) are shown on Fig. 4. In both cases, a first order relation ($R^2 \geq 0.97$) was found between the number of CV stimulation and the residual DEX amount [Eq. 1, where M_r (μg) is the residual quantity of DEX in the film after n cycles, M_r^0 (μg) is the initial quantity of DEX in the film and k is a constant (μg/#cycle)].

$$\frac{dM_r}{dn} = -k \cdot M_r \Leftrightarrow \ln M_r = \ln M_r^0 - k \cdot n \quad (1)$$

Here, M_r^0 was assumed to be equal to the released DEX amount after 150 CV stimulation scans. This represents 39 and 106 μg/cm^2 for the thin and thick samples, respectively. Based on previous reported results [12], this is a sufficient amount of release DEX to significantly reduce the inflammatory tissue reaction in the vicinity of the electrode. As a control, PPy/DEX coated electrodes were simply immersed in PBS and the amount of DEX liberated was measured in function of time (so-called *passive release*). As expected and shown on Fig. 4, the amounts of DEX released by simple diffusion (passive release) were very low and negligible compared to the amounts released by electrical stimulation (assuming that 1 CV at 100 mV/s



Scheme 1 Reduction (**a**) and oxidation (**b**) reactions of a PPy/DEX film during electrical stimulation

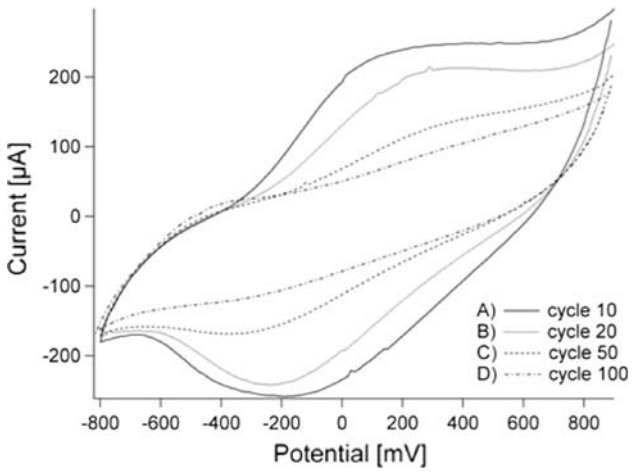


Fig. 3 Cyclic voltammograms of a nanostructured PPy-DEX film (Q_{PPy} = 39 mC/cm^2) recorded after 10, 20, 50 and 100 cycles of electrical stimulation. Scan rate = 100 mV/s

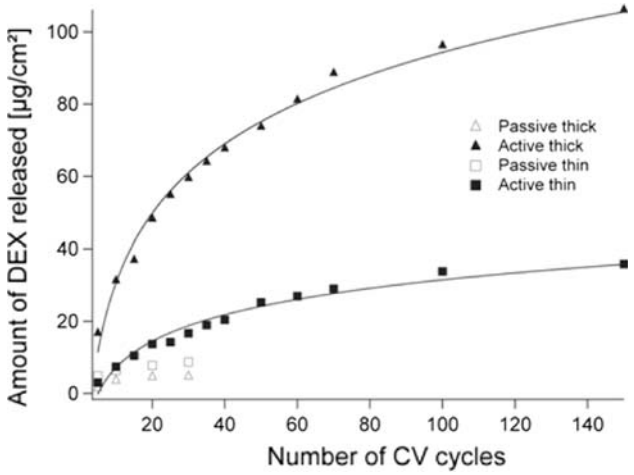


Fig. 4 Release profiles of DEX from thin (*filled square*, Q_{PPy} = 27.4 mC/cm^2) and thick (*filled triangle*, Q_{PPy} = 700 mC/cm^2) nanostructured PPy films, determined by UV spectroscopy at 242 nm. The drug release follows a first order kinetics. The *x* axis represents the number of CV cycles at 100 mV/s for the active release and the time for the passive release (assuming that 1 CV takes ~1 min at 100 mV/s)

corresponds to ~1 min). This indicates that our nanostructured PPy based drug release electrodes are true electrically driven release systems.

In order to study the influence of the PPy film properties on the release behavior of the nanostructured electrodes, samples with PPy/DEX films of various thicknesses were synthesized. As shown on Fig. 4, the released DEX amount increases with the film thickness, but the release profile remains identical. However, the quantity released does not linearly correlate with the film thickness. Here, the PPy/DEX films were synthesized with the same current density, but a 25 times increase of the electrical charge (from 27 mC/cm^2 for the thin sample to 700 mC/cm^2 for the thick sample) corresponds to only a three times increase of the liberated DEX amount after 150 CV cycles. This can be due to the fact that PPy releases drug most efficiently from its surface, as opposed to from within the polymer bulk at the chosen rather high CV scan rate (100 mV/s). We, indeed, observed that the scan rate influences the release profile (data not shown). When decreasing the scan rate, the released DEX amount per cycle increased, and the film was more rapidly drained of DEX.

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

The morphology of PPy/DEX films on nanostructured electrodes were observed by SEM after 150 CV stimulating cycles of release. Unlike conventional planar PPy films that show cracks and delamination after a few number of cycles [12], the surface of nanostructured PPy/DEX films appear similar before and after DEX release (Fig. 2). This improvement of the mechanical stability of the PPy film and of the adherence between the polymer and the metallic electrode substrate, that should help to lengthen the lifetime of the implanted electrode, can be attributed to the better anchoring of PPy film by the Pt nanopillars. Finally, we should mention that though the initial electroactivity of PPy/DEX film is increased by the nanostructuration of the metallic electrode substrate, we still observe, as on planar metallic electrodes, a decrease of the electroactivity upon DEX release (Fig. 3b–d). This progressive loss of electroactivity probably occurs partly through the loss of doping molecules, but it is also probable that the stimulation over this range of potential induce a progressive overoxidation of the film, leading to a loss of its electroactivity. We are, therefore, currently trying to optimize the synthesis parameters in order to get stable electrochemical properties of PPy films over time, as this is a required condition for prolonged release applications.

4 Conclusion

We have developed a method allowing the easy and reproducible fabrication of switchable PPy-based nanobioelectrodes for local controlled delivery of anti-inflammatory dexamethasone molecules. The drug incorporation was done using a one-step electropolymerization of a PPy film onto an electrode covered by a brush of metallic nanopillars. The release of the drug was carried out using cyclic voltammetry stimulus and was found to be electrically controllable. Due to the increased surface active area, the actuation redox potentials of nanostructured PPy were

found to be lower than on conventional planar PPy films. Moreover, the presence of the Pt nanopillars onto the electrode substrates improves the mechanical stability and the adherence of the polymer onto the metallic substrate. Both of these parameters are of great importance for the development of long-term performances neural stimulation electrodes. However, further improvements of electrochemical stability and drug releasing properties are expected by optimizing various synthesis parameters and electrical stimuli. Though, in this work, we studied the delivery of DEX, the nanobioelectrodes can be easily adapted for the delivery of a wide range of biomolecules and drugs, and are therefore expected to be of interest for a variety of electrode/tissue interfaces in biomedical devices.

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References

1. Fawcett JW, Asher RA. The glial scar and central nervous system repair. Brain Res Bull. 1999;49:377–91.
2. Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods. 2005;148:1–18.
3. Shain W, Spataro L, Dilgen J, Haverstick K, Retterer S, Isaacson M, et al. Controlling cellular reactive responses around neural prosthetic devices using peripheral and local intervention strategies. IEEE Trans Neural Syst Rehabili Eng. 2003;11:186–8.
4. Spataro L, Dilgen J, Retterer S, Spence AJ, Isaacson M, Turner JN, et al. Dexamethasone treatment reduces astroglia responses to inserted neuroprosthetic devices in rat neocortex. Exp Neurol. 2005;194:289–300.
5. Koehler PJ. Use of corticosteroids in neuro-oncology. Anticancer drugs. 1995;6:19–33.
6. Kaal EC, Vecht CJ. The management of brain edema in brain tumors. Curr Opin Oncol. 2004;16:593–600.
7. Zhong Y, Bellamkonda RV. Biomaterials for the central nervous system. Brain Res. 2007;1148:15–27.
8. George PM, Lyckman AW, LaVan DA, Hegde A, Leung Y, Avasare R, et al. Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. Biomaterials. 2005;26:3511–9.
9. Pernaut JM, Reynolds JR. Use of conducting electroactive polymers for drug delivery and sensing of bioactive molecules. A redox chemistry approach. J Phys Chem B. 2000;104:4080–90.
10. Richardson RT, Thompson B, Moulton S, Newbold C, Lum MG, Cameron A, et al. The effect of polypyrrole with incorporated neurotrophin-3 on the promotion of neurite outgrowth from auditory neurons. Biomaterials. 2007;28:513–23.
11. Richardson RT, Wise AK, Thompson BC, Flynn BO, Atkinson RJ, Fretwell NJ, et al. Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons. Biomaterials. 2009;30:2614–24.
12. Wadhwa R, Lagenaur CF, Cui XT. Electrochemically controlled release of dexamethasone from conducting polymer polypyrrole coated electrode. J Control Release. 2006;110:531–41.
13. Thil M-A, Delbeke J, Colin I, Magnin D, Ferain E, Demoustier-Champagne S. Drug eluting nanowire array. European patent pending no EP-07118428.7 (2007).
14. Duvail JL, Dubois S, Demoustier-Champagne S, Long Y, Piraux L. Physical properties of magnetic metallic nanowires and conjugated polymer nanowires and nanotubes. Int J Nanotechnol. 2008;5:838–50 (and references cited there in).
15. http://www.it4ip.be.
16. Luo X, Cui XT. Electrochemically controlled release based on nanoporous conducting polymers. Electrochem Commun. 2009;11:402–4.