

Effect of Pyrrole Implants Synthesized by Different Methods on Spinal Cord Injuries of Rats

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ABSTRACT

Polypyrrole (PPy) and polypyrrole/polyethylene glycol (PPy/PEG) implants synthesized by chemical, electrochemical, and plasma polymerization methods were implanted into the injured spinal cord of rats to determine their effect on motor function recovery. Before implantation, the materials were characterized by infrared (IR) spectroscopy. An experimental model of traumatic spinal cord injury (TSCI) by complete transection at thoracic level 9, in rats was used. The polymer implants were inserted immediately after transection. Motor function recovery was evaluated once a week during 5 weeks using the Basso, Beattie and Bresnahan (BBB) motor scale. Histological evaluation was done at the end of the recovery evaluation period using hematoxylin/eosin stain. Results showed that animals implanted with polymers synthesized by plasma had a better integration into the nerve tissue, less inflammatory response and a better functional recovery than animals implanted with polymers synthesized by chemical or electrochemical methods.

Keywords: fpolypyrrole implants, chemical synthesis, electrochemical synthesis, plasma synthesis, traumatic spinal cord injury.

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RESUMEN

En el presente trabajo se comparó el efecto de implantes poliméricos derivados del pirrol (polipirrol o PPy) y del copolímero polipirrol/polietilenglicol (PPy/PEG), obtenidos por diferentes métodos de síntesis: químico, electroquímico y polimerización por plasma con el propósito de determinar si el método de síntesis puede influir sobre el efecto que producen al ser implantados después de una lesión traumática de la médula espinal de ratas. Antes de realizar el implante, las características químicas y estructurales de los polímeros fueron analizadas por espectroscopia de infrarrojo (IR). Se utilizó un modelo experimental de lesión traumática de médula espinal (LTME) por sección completa en ratas. La LTME se realizó a nivel torácico 9 y el polímero fue implantado de inmediato en la zona de lesión. La recuperación de la función motora se evaluó mediante la escala Basso, Beattie y Bresnahan (BBB) una vez por semana durante 5 semanas. La evaluación histológica se realizó al término del seguimiento con la tinción de hematoxilina/eosina. Los resultados muestran que los animales implantados con polímeros sintetizados por plasma se integraron mejor al tejido nervioso, redujeron la respuesta inflamatoria y favorecieron una mayor recuperación funcional en comparación con los animales implantados con materiales sintetizados por métodos químicos o electroquímicos.

Palabras clave: implantes de polipirrol, síntesis química, síntesis electroquímica, síntesis por plasma, lesión traumática de la médula espinal.

INTRODUCTION

Traumatic spinal cord injuries (TSCI) trigger a series of secondary events that increase the original damage, prevent axonal regeneration and produce different degrees of functional impairment below the site of injury which can lead to permanent paralysis [1].

To date, due to the complex pathophysiology of TSCI, no therapeutic strategy has been effective to restore the lost functions after lesion. Many strategies have been suggested and attempted in order to find a solution to this problem. One proposal in this field consists of using transplants of tissues or cells to promote axonal regeneration and functional recovery after TSCI [2]. Among experimental transplants that have produced positive results are fetal tissue [3], fresh or predegenerate peripheral nerve [4-5], Schwann cells alone or in combination with different molecules [2,6], olfactory ensheathing cells [2,7] and neural stem cells [2,8-9].

Notwithstanding, the majority of the transplants that have been used in the treatment of TSCI have not been able to restore the nerve function in a significant way. Recently, research in tissue engineering has produced materials that have the potential of being a better treatment for this pathology [10-12].

To form a viable transplant for TSCI treatment, the materials employed must be biocompatible with the nervous tissue (where the communication is mainly by ion exchange), and their chemical composition, hydrophobicity and electrical activity should be well characterized.

Recently, polymers with intrinsic conductive properties have gained relevance as smart materials with biological applications [13]. These polymers possess the physical and chemical properties of organic polymers and the electrical properties of the metals [14]. Additionally, these materials have the ability to bind to various chemical substances (dopants) which

affect the physicochemical properties of the polymers, such as their conductivity. Furthermore, materials of this type have been developed with the capability of supporting and modulating the growth of different cell types which makes them suitable for biological and biomedical applications [15].

Among biocompatible and electrically conductive polymers, polypyrrole (PPy) is one of the most studied. PPy is easy to synthesize, thermally stable, and has high conductivity in comparison with other conductive polymers [16]. PPy has been used as biosensor for measuring cholesterol [17], glucose in blood [18], blood group, antibodies [19], and vapors of organic solvents [20]. It has also been used for coating neural probes [21], devices for drug and biomolecule release [22], and artificial muscles [23]. In addition to being biocompatible [24], the cytocompatibility of PPy has been demonstrated using L929 mouse fibroblast and Neuro2a neuroblastoma cells [25]. Moreover, PPy has been used to support cell adhesion and growth of different cell types *in vitro* [13, 26-34], and as guide for regenerating rat sciatic nerve [33, 35]. Our research group, has demonstrated that PPy synthesized by plasma polymerization promotes neuroprotection and leads to recovery of motor and sensory functions after a TSCI by complete section in *in vivo* studies with rats [36-38]. These PPy implants showed no significant inflammatory response *in situ* after 4 weeks of implantation [25].

Typically, PPy is synthesized by chemical and electrochemical polymerization methods. It is also possible to synthesize it by plasma polymerization, but the product shows important differences. The chemically synthesized PPy is crystalline, and there is a lack of control over the mass or the thickness of the film obtained [39]. PPy synthesized electrochemically, has similar chemical structure to that obtained by chemical methods, but can be more easily doped to enhance its conductive properties. Also, the film properties can be controlled

directly in the course of the polymerization. Both the chemical and electrochemical synthetic methods use accelerators and solvents, which modify the adhesion and hydrophobicity properties of the resulting materials. These extra components can be dangerous or toxic; thus they must be fully removed from the polymers before being applied to any biological system. In contrast, polymerization by plasma only requires the base monomer to start the reaction without introducing other chemical compounds. When plasma polymerization is used, the oxidation is promoted by the impact of free electrons that travel along the electric field with monomer molecules [40]. The material produced by plasma polymerization does not have a regular chemical structure but specific chemical groups are present. Plasma polymerization produces a dense film with a crosslinked structure [41]. The crosslinked structure permits the material to retain its mechanical properties even in biological media, while the functional groups exposed to the surface mediate favorable interactions with many types of cells.

Given the differences in chemical and physical structure of the PPy materials obtained by different synthesis methods, it is important to evaluate how these variations affect implants and their effect on recuperation from spinal cord injuries. The goal of the present study is to compare implants made of pyrrole derivatives, PPy and PPy co-polymerized with polyethylene glycol (PPy/PEG) obtained by chemical, electrochemical and plasma polymerization. We analyze the chemical structure of the prepared implants, their ability to integrate with nervous tissue, their inflammatory response and their effect on the recovery of motor function after a TSCI in rats. In this study PPy/PEG was tested because after a TSCI the neuronal membranes are ruptured and PEG has been shown to aid in the repair of membranes [42-43]; which could enhance the benefic effect of PPy when implanted after a TSCI.

MATERIALS AND METHODS

Plasma polymerization

The preparation of iodine doped PPy (PPy/I) and PPy/PEG thin films has been previously described [36-37]. Briefly, the films were prepared in a tubular glass reactor, 9 cm in diameter and 25 cm long, capped with stainless steel flanges with access ports. The ports were used to connect the reactor to a vacuum pump, a Pirani gauge (Edwards), and to introduce reactants. For the preparation of PPy films, pyrrol (Aldrich, 99%) vapor was used; and to synthesize the PPy/PEG copolymer, pyrrol was introduced by one port and, simultaneously, PEG (Aldrich) was introduced through another port. In the center of the flanges two stainless steel flat circular electrodes with 7 cm of diameter and separated by 9 cm, were inserted with the aim to create a homogenous electric field in the reactor. The electric field was generated by a Dressler Cesar 136 RF Power Generator. A field frequency of 13.5 MHz and power of 18 W was used. The pressure in the reactor was 5×10^{-2} Torr and the synthesis time was 300 min. The polymers were separated from the internal reactor walls applying acetone and using a thin spatula.

Electrochemical synthesis

PPy/PEG copolymer was synthesized by the conventional electrochemical method using an titanium electrode (99.99% Sigma-Aldrich) with an exposed area of 4.0 cm². Before each experiment, the electrode surface was polished with silicon carbide (SiC) paper grade 2000, degreased with acetone and rinsed with deionized water. For the electropolymerization an aqueous solution of 0.2 M pyrrol monomer in 0.2 M of oxalic acid (Ac. Ox) with 8% PEG (molecular weight 600 Daltons) was prepared. All solutions were prepared with deionized water (18.2 MΩ).

Electrochemical polymerizations were performed at room temperature in a

conventional cell with three electrodes: a rod of Ti functioned as the active electrode, a saturated calomel electrode (SCE) that functioned as the reference electrode, a rod of platinum (Pt) as the counter electrode. All electrodes were connected to a Potentiostat galvanostat Autolab PGSTAT 302N with the GPES 4.9 electrochemical software.

The PPy/PEG film was synthesized potentiostatically at 0.9 V vs. SCE, and allowed to grow for 4 hours. The film was rinsed with deionized water and removed from the Ti electrode surface and left to dry at a temperature of 60 °C for 24 hrs.

Chemical synthesis

Two different chemically synthesized PPy were used. A commercial PPy doped with sulphonic acid was purchased from Sigma-Aldrich (CAS 30604-81-0 and 577030-5G, Pcode 1000874358, Lot # MKB, elemental analysis C-69.94 %; N-15.42 % (N/C=0.22), O-12.58 %; S-2.06 %).

The other PPy was synthesized in our laboratory by oxidation. The polymer was synthesized by mixing 48 mL of dodecylbenzenesulfonic acid (70 wt. % dissolved in 2-propanol), 900 mL of distilled water, 7 mL of pyrrole, and 11.4 g of ammonium persulphate dissolved in 25 mL of water. The reaction was performed at 25° C during 24 hours.

Infrared Characterization

The polymers were analyzed by infrared (IR) spectroscopy with a Nicolette 550 spectrophotometer with a 400-4000 cm⁻¹ interval using 32 scans [36, 40].

Implants

Each polymer was pulverized and then compressed at 9 Ton/cm² for 10 min to form a thin tablet of 1 cm in diameter and 0.5 mm thickness. Finally, the thin table was cut according to the diameter of the spinal cord.

Animals and surgical procedures

Female Long Evans rats with 220 to 260 g of body weight were maintained under standard laboratory conditions and free access to food and water. Animal care and the protocols for animal use were approved by the Scientific and Ethics Committees of the Instituto Mexicano del Seguro Social.

Eighteen rats were prepared to receive a complete spinal cord section at thoracic 9 level (T-9) and then, six experimental groups (n=3 animals per group) were formed:

1. Control: animals without implant.
2. PPy: animals implanted with PPy synthesized by plasma.
3. ChPPy1: animals implanted with PPy chemically-synthesized.
4. ChPPy2: animals implanted with PPy purchased from Sigma-Aldrich (chemically-synthesized).
5. PPy/PEG: animals implanted with PPy/PEG synthesized by plasma.
6. EPPy-PEG: animals implanted with PPy/PEG electrochemically-synthesized.

Before implantation, animals were anesthetized intramuscularly with a mixture of ketamine and xylazine (77.5 and 12.5 mg/kg). Then, an aseptic surgery under microscopic inspection was done by making a sagittal incision on the skin from the middle back followed by a dissection of the spine paravertebral muscles. Two laminae were removed (T-8 and T-9) to expose the spinal cord tissue [36-38].

The meninges were longitudinally cut and spinal cord tissue was completely transected by cutting transversally all fibers. Transection was corroborated with a microsurgical hook to ensure that no pathway remained connected. The corresponding implant (approximately 10 mg) was then

inserted at the injured zone in the cavity between both sides of the transection. Only the animals in the control group did not receive any implant. Finally, the meninges were sutured, as well as the paravertebral muscles and skin. Animals were treated with an anti-inflammatory drug (0.31ml/62.5ml of paracetamol into drinking water during 3 days) and an antibiotic (200 μ L of benzathine penicillin, in one i.m. dose). Afterwards, rats recovered from anesthesia and surgical procedures in an intensive care unit for small animals (Schoer Manufacturing CO., Kansas City, MO, USA) and placed into individual acrylic cages with sterile sawdust for receiving food and water *ad libitum*. The day after SCI, the absence of hind limb movement was corroborated to ensure the complete section of the spinal cord. Their intestine and bladder were handled by manual expression twice a day and visual inspection was performed day by day looking for skin irritation or *decubitus* ulcers [36-38].

Motor function recovery

The motor function recovery of hind limbs in each rat from the six groups were assessed weekly during five weeks using the Basso, Beattie and Bresnahan (BBB) scale [44], which has 22 points and where 0 represents total absence of movement (paralysis) and 21 represents a normal walking. The test was applied by two observers blinded to the treatment that the animals received.

Histological analysis

Thirty days after spinal cord transection, all animals were anesthetized as described before, followed by intraperitoneal administration of 0.2 mL of heparin. Then, a wide thoracotomy was performed and 200 mL of cool physiological saline solution was perfused transcordially at 30 mL/min followed by 400 mL of 4% paraformaldehyde in phosphate buffer. Afterwards, 2 cm of the spinal cord were taken including the

injury zone. The spinal cord specimens were embedded in paraffin. Serial longitudinal 10 μm thick sections were obtained and stained with hematoxylin and eosin. Images were obtained using a computerized system equipped with an IM 1000 software and a 300 FX digital camera [36-38].

Statistical analysis

The BBB scores were evaluated using ANOVA of repeated measures followed by Dunnett's test. Significant differences were considered when $p < 0.05$. All analyses were performed using the SPSS 16.0 software.

RESULTS

Chemical structure of implants

The chemical structure of polymers employed in the experiment was determined by IR spectroscopy. Figure 1 shows the similarity between the two chemically-synthesized PPy; in particular in the region between 1750 and 4000 cm^{-1} . Small differences in the spectra can be observed between 400 and 1750 cm^{-1} which we believe are due to some substitutions in the pyrrole rings. In contrast, the same figure shows a significant difference between the chemically-synthesized PPy and PPy synthesized by plasma. At 3355 cm^{-1} one can observe the different vibrations of the primary and secondary amine groups. Another difference is the vibration corresponding to nitrile groups at 2213 cm^{-1} .

Figure 2 shows the IR spectra of PPy/PEG copolymers synthesized electrochemically and by plasma polymerization. It can be observed that only the four absorptions highlighted in green coincide in both materials. In the copolymer synthesized by plasma, the main absorptions are in 717, 1617, 2212, 2928 and 3312 cm^{-1} ; it is notable that none of these absorptions were identified in the electrochemically-synthesized copolymer, which shows main absorptions at 664, 2360 and 1033 cm^{-1} .

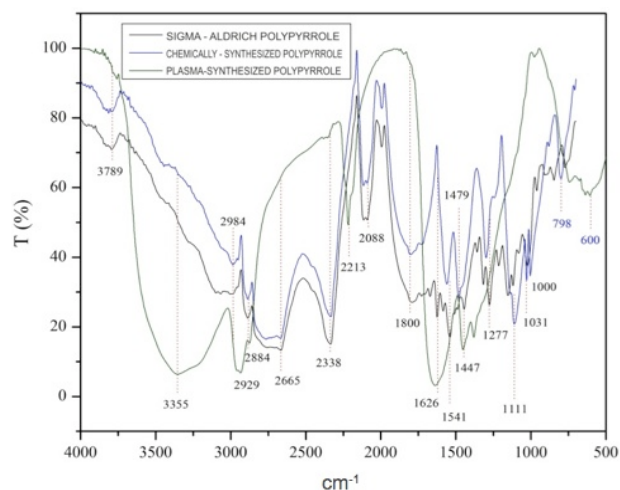


Figure 1. IR spectra of PPy. Chemically-synthesized PPy (blue line), Sigma-Aldrich PPy (black line) and plasma-synthesized PPy (green line).

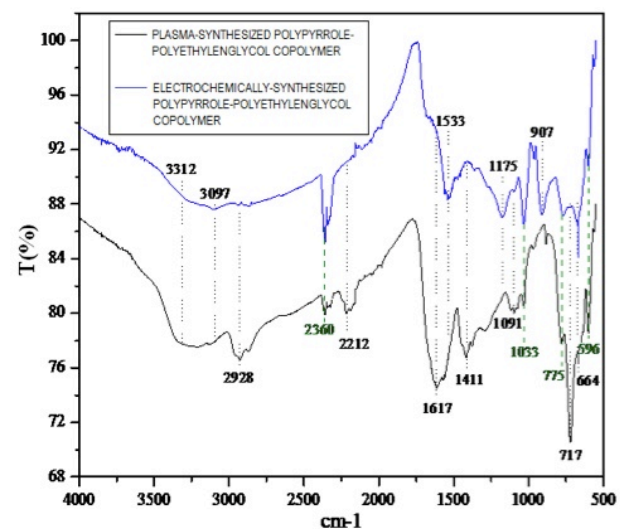


Figure 2. IR spectra of PPy/PEG synthesized by plasma polymerization (black line) and electrochemically-synthesized (blue line). Absorptions found in both copolymers are highlighted in green.

These differences are indicative of a significant difference in chemical structure between the two copolymers.

In the copolymer synthesized by plasma, the more intense absorption was found at 717 cm^{-1} and corresponds to =C-H groups, the absorption at 1617 cm^{-1} can be associated with C = C groups, the absorption at 2212 cm^{-1} correspond to nitrile groups, while the absorption found at 2928 cm^{-1} indicates the presence of -CH aliphatic groups which

can arise from ethylene glycol segments or from fragments of pyrrole molecules which were fractionated due to the high energy of the particles in the plasma, the absorption at 3312 cm^{-1} can be associated with the presence of amine groups. These absorptions indicate that in the copolymer synthesized by plasma, the heteroaromatic character of the pyrroles predominates.

In the case of the electrochemically-synthesized co-polymer, the more intense absorption was found in 664 cm^{-1} and can be assigned to N-O groups. This implies a substitution of N-H groups by N-O groups which could be a consequence of ethylene glycol participation during the synthesis. The absorption at 1033 cm^{-1} corresponds to C-O groups that are found in ethylene glycol repeat units. These absorptions indicate

that in the electrochemically-synthesized copolymer, the oxygenated character of the ethylene glycol predominates.

Motor function recovery

The motor function was evaluated 24 h after complete transection of the spinal cord with the aim to corroborate if hind limb performance showed complete bilateral paralysis in all rats (BBB=0). Afterwards, the BBB locomotor rating scale was assessed once a week during 5 weeks to evaluate the gradual improvement of the animals. Results were as follow (Figure 3):

- a) Control (3 animals alive): BBB=1, meaning that animals had slight movement of 1 or 2 joints.

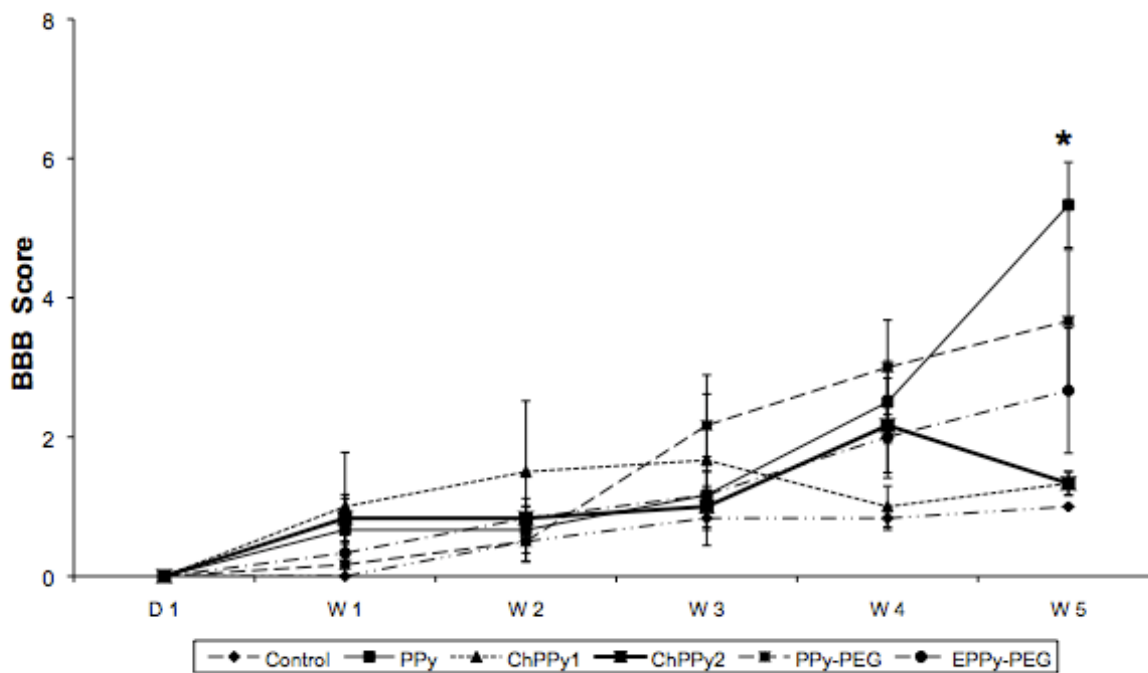


Figure 3. The locomotion recovery measured by BBB open field score after traumatic spinal cord injury was evaluated along the time. Control: animals without implant; PPy: animals implanted with polypyrrole (PPy) synthesized by plasma; ChPPy1: animals implanted with PPy chemically-synthesized; ChPPy2: animals implanted with PPy purchased from Sigma-Aldrich (chemically-synthesized); PPy/PEG: animals implanted with PPy copolymerized with polyethylene glycol (PPy/PEG) and synthesized by plasma; EPPy/PEG: animals implanted with electrochemically-synthesized PPy/PEG co-polymer. Results are expressed as means \pm SE. ANOVA of repeated measures followed by Dunnett's test. *Control group different from PPy and PPy/PEG ($p < 0.028$, and 0.05 , respectively).

- b) PPy (3 animals alive): BBB=5.33, meaning that animals were able to move the 3 joints of the hind limb (hip, knee and ankle), and in two them, the movements were extensive.
- c) ChPPy1 (3 animals alive and 2 dead animals, non-evaluable): BBB=1, meaning that the animals had slight movement of 1 or 2 joints. Although one of the animals had an improvement of 4 points in the BBB scale in the third week, score decreased with time.
- d) ChPPy2 (3 animals alive and 4 dead animals, non-evaluable): BBB=1.33, meaning that the animal had slight movement in one or two joints. But just as in the ChPPy1 group, one animal presented improvement until the fourth week and subsequently decreased the effect of the implant.
- e) PPy-PEG (3 animals alive): BBB=3.66, meaning that animals had extensive movement in 1 or 2 joints of the hind limb and slight movement of other joint (hip, knee and ankle).
- f) EPPy-PEG (3 animals alive): BBB=2.6, meaning that animals had extensive movement of 1 joint and slight movement of 2 joints of the hind limbs.

The dead animals did not show infection of the urinary or respiratory tract, hypertrophy of urethral *meatus*, or shallow or deep wounds. Thus, the cause of death is unknown.

Animals implanted with materials synthesized by plasma demonstrated greater motor function recovery comparing with animals implanted with materials obtained by chemical or electrochemical synthesis and with control group animals. Animals with implants synthesized by plasma showed significant differences with animals from the control group; PPy ($p = 0.028$) and PPy / PEG ($p = 0.05$).

Histological analysis

Four weeks after SCI, animals were sacrificed to analyze the integration of implants to the spinal cord tissue and the inflammatory response. The control group (Fig. 4A) showed greater tissue destruction compared with implanted animals. Analysis of implants integration to the spinal cord tissue showed that PPy implants synthesized by plasma polymerization were well integrated to the tissue and that the surrounding tissue showed little destruction (Fig. 4B). The chemically-synthesized implants ChPPy1 and ChPPy2 (Fig. 4C and 4D respectively) had similar amount of tissue destruction and complete lack of implant integration, with a great cyst at the injury epicenter and a complete loss of histological architecture.

Regarding the inflammatory response, morphometric analysis showed the presence of 142 polymorphonuclear cells per every 4 microns in the animals with ChPPy1 implant and 128 polymorphonuclear cells in the animals with ChPPy2 implant (obtained from Sigma-Aldrich). In contrast, the morphometric analysis of samples with implants synthesized by plasma for both PPy and PPy/PEG showed about 74 inflammatory cells at the tissue surrounding the implant, and if PPy was doped with iodine (PPy/I) 95 inflammatory cells were found in the tissue that surrounded the implant.

DISCUSSION AND CONCLUSION

The present work compared the effect of different implants derived from pyrrole (PPy and PPy/PEG) and obtained by standard chemical, electrochemical, and plasma polymerizations. The implants were analyzed according to their chemical structure, integration with the nervous tissue, and their effect on functional recovery in rats with complete section of the spinal cord.

The PPy implants that were chemically synthesized as ChPPy1 and ChPPy2 have

a similar chemical structure. None of them were integrated to the nervous tissue, a large number of cysts and inflammatory cells were found on the tissue around the implants, and a complete loss of cytoarchitecture of the spinal cord was observed. Animals that received ChPPy1 or ChPPy2 showed some functional recovery between the third and fourth week post injury. This recovery, however, decreased afterwards. Meanwhile, animals implanted with PPy synthesized by plasma showed implant integration to the nerve tissue, little destruction of nervous tissue and significant functional recovery when comparing with animals with implants obtained by chemical methods and animals from control group ($p = 0.028$).

Although both implants of PPy copolymerized with PEG and synthesized by plasma or electrochemically were well integrated to the nervous tissue, the functional recovery was different. Animals that received implants of PPy/PEG synthesized by plasma showed greater motor recovery ($p = 0.05$ with respect to animals from control group) than those which received implants obtained electrochemically.

The results show significant differences in the effects of the method of synthesis on functional recovery and implant integration to the nervous tissue. Although the polymers had the same molecules of origin, IR analysis showed that method of synthesis affects the chemical structure of the material.

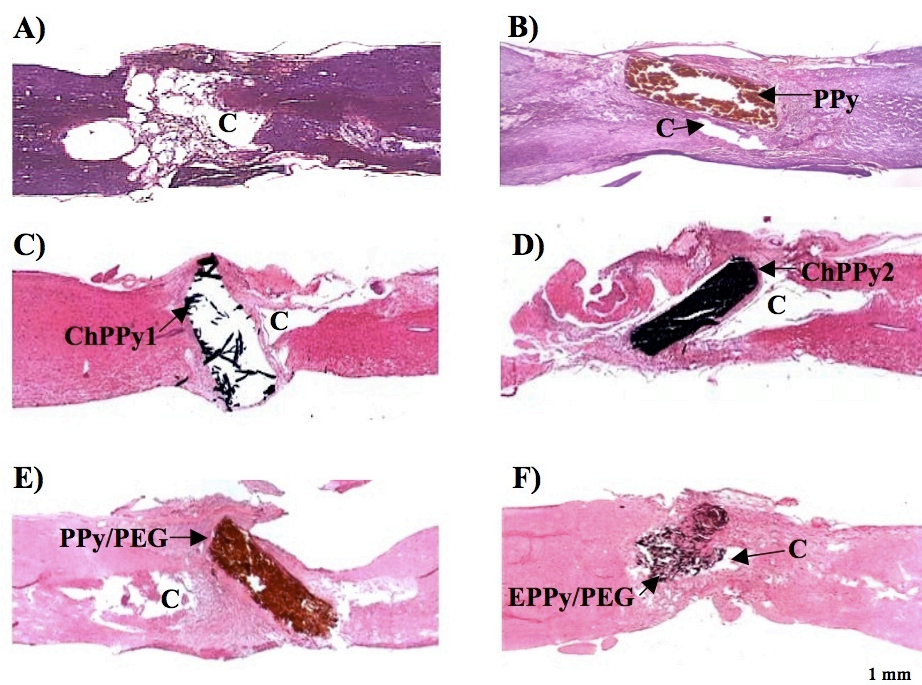


Figure 4. Representative photomicrographs from longitudinal sections of spinal cord of rats that showing the epicenter zone of injured spinal cord by complete transection a) Control: animals without implant; b) PPy: animals implanted with PPy synthesized by plasma; c) ChPPy1: animals implanted with PPy chemically-synthesized; d) ChPPy2: animals implanted with PPy purchased from Sigma-Aldrich (chemically-synthesized); e) PPy/PEG: animals implanted with PPy/PEG copolymer synthesized by plasma-; f) EPPy/PEG: animals implanted with electrochemically-synthesized PPy/PEG copolymer. Magnification 2X. Hematoxylin/eosin stain. C→Cyst, PPy, ChPPy1, ChPPy2, PPy/PEG and EPPy/PEG→Implants.

Wangh et al., showed that the chemical structure of PPy and of polythiophene synthesized by plasma are different from the ones chemically synthesized because the materials obtained by plasma are highly crosslinked and branched [45] and they can form three-dimensional network which can support and promote neural cells grown while the presence of heteroaromatic amine groups and nitrile groups can favor neuroprotection after an injury in the central nervous system. Furthermore, the polymers synthesized by plasma are insoluble, thermally stable and chemically inert [46], characteristics that make them more desirable for implants in a biological system.

Pyrrole belongs to a class of heterocyclic compounds that are in various natural components such as the heme group, chlorophyll and vitamin B12 [47] and have been used in various applications of chemical medicine as anti-inflammatory, antibacterial and antihypertensive agents, as well as agents for tyrosine kinase inhibition[48].

Currently, PPy is mainly synthesized by chemical oxidation or electrochemical polymerization [16]. Nevertheless, for biomedical applications, plasma synthesis might be a better option because the unique characteristics of PPy generated by plasma that allow improvement of the cellular microenvironment and favor the attachment and growth of cells. The low rate of degradation of the material maintains the cell adhesion, promotes morphological maturation and allows preservation of its properties [34]. It has been shown in cultures of nerve cells on surfaces treated with PPy, that the adhesion, proliferation, attachment, viability and number of synapses is increased compared with that observed in cultures performed on surfaces treated with compounds as Poly DL-Ornithine / Laminin [34]. Furthermore, our research group has demonstrated neuroprotective activity of PPy synthesized by plasma, as well as greater functional recovery in animals that received

this type of implant after a TSCI, compared with those who did not receive it [36-38].

The above may be due to the chemical structure of PPy obtained by plasma polymerization. IR spectroscopy of this material shows the presence of a variety of chemical groups including primary amines, nitriles and aliphatic sections [34], and it has been shown that the structures consisting mainly of methyl-, hydroxyl-, amino- and carboxyl- functional groups, which are found in natural biological surfaces, favor the growth of cells [49].

The adaptability of tissues to materials such as the implants studied in this work begins with the absorption of solutions at the surface of the material. The hydrophilic properties of PPy, which can be increased by increasing the ionization capability of the material, allow one to store solutions and favor interaction with cells [50-51], which generates optimal sites for cell attachment.

Nerve cells carry out their function by generating electrical activity. The nervous system thus responds to electric fields and the key component of the neural communication is the action potential generated in the synapse. This implies that the ideal biomaterial to implant in this system must introduce electrical stimulators to promote neuronal growth and nerve regeneration [13]. The material must promote regeneration of the nervous tissue at the interface by attraction or rejection of ions and polar groups between the cells and the material. In addition to this, it has been shown that electrical stimulation alters the absorption of proteins and the interaction with the nerve cells [52], which could also favor nerve regeneration processes. Although the breaking of rings in the plasma polymerization process results in a polymer complex of low conductivity, when is introduced into a biological system its sensitivity to humidity increases its conductivity [40]. It is known that the electric conductivity of conductive polymers

synthesized by chemical or electrochemical methods oscillates within a range of 10^{-10} to 10^{-5} S cm^{-1} [39]. The electric conductivity of the plasma-synthesized PPy measured at 30 % relative humidity is around 10^{-12} S cm^{-1} , while at 90 % of relative humidity it is 10^{-9} S cm^{-1} [40]. The PPy/PEG copolymer has a conductivity of 10^{-12} S cm^{-1} at 30 % of relative humidity [36] and of 10^{-9} to 10^{-8} S cm^{-1} when it is dampened with ionic solutions [51].

Due to their physical and chemical properties, the PPy does not alter the biological functions of the cell cultures and provides better cell attachment and an increased rate of proliferation, which may be due to the accumulation of amino groups (-NH₂) and interaction with other groups generated during the process of plasma polymerization [34], which may explain the better results obtained when using PPy synthesized by plasma vs the PPy synthesized by conventional chemical or electrochemical methods.

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