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BIOCOMPATIBILITY STUDIES

Original Research

Functional recovery in spinal cord injured rats using polypyrrole/iodine implants and treadmill training

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Abstract Currently, there is no universally accepted treatment for traumatic spinal cord injury (TSCI), a pathology that can cause paraplegia or quadriplegia. Due to the complexity of TSCI, more than one therapeutic strategy may be necessary to regain lost functions. Therefore, the present study proposes the use of implants of mesoparticles (MPs) of polypyrrole/iodine (PPy/I) synthesized by plasma for neuroprotection promotion and functional recovery in combination with treadmill training (TT) for neuroplasticity promotion and maintenance of muscle tone. PPy/I films were synthesized by plasma and pulverized to obtain MPs. Rats with a TSCI produced by the NYU impactor were divided into four groups: Vehicle (saline solution); MPs (PPy/I implant); Vehicle-TT (saline solution + TT); and MPs-TT (PPy/I implant + TT). The vehicle or MPs (30 μL) were injected into the lesion site 48 h after a TSCI. Four days later, TT was carried out 5 days a week for 2 months. Functional recovery was evaluated weekly using the BBB motor scale for 9 weeks and tissue protection using histological and morphometric analysis thereafter. Although the MPs of PPy/I increased nerve tissue preservation ($P = 0.03$) and promoted functional recovery ($P = 0.015$), combination with TT did not produce better neuroprotection, but significantly improved functional results ($P = 0.000$) when comparing with the vehicle group. So, use these therapeutic strategies by separately could stimulate specific mechanisms of neuroprotection and neuroregeneration, but when using together they could mainly potentiate different mechanisms of neuronal plasticity in the preserved spinal cord tissue after a TSCI and produce a significant functional recovery.

Graphical Abstract The implant of mesoparticles of polypyrrole/iodine into the injured spinal cord displayed good integration into the nervous tissue without a response of rejection, as well as an increased in the amount of preserved tissue and a better functional recovery than the group without transplant after a traumatic spinal cord injury by contusion in rats. The relevance of the present results is that polypyrrole/iodine implants were synthesized by plasma instead by conventional chemical or electrochemical methods. Synthesis by plasma modifies physicochemical properties of polypyrrole/iodine implants, which can be responsible of the histological response and functional results. Furthermore, no additional molecules or trophic factors or cells were added to the implant for obtain such results. Even more, when the implant was used

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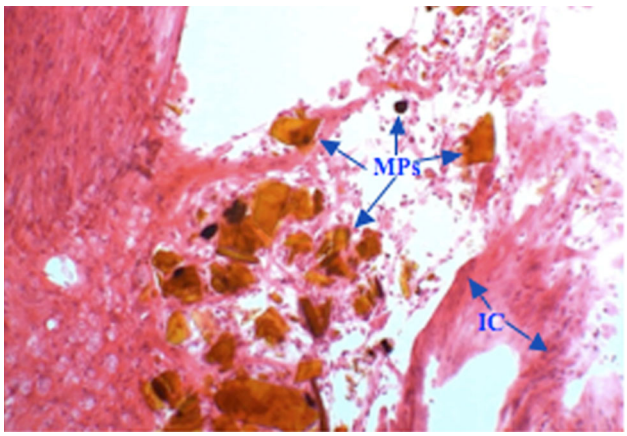
together with physical rehabilitation, better functional recovery was obtained than that observed when these strategies were used by separately.



1 Introduction

Traumatic spinal cord injury (TSCI) is one of the most significant health problems worldwide because it produces paraplegia or quadriplegia and a broad range of secondary complications [1]. To date, TSCI remains a devastating condition with no universally accepted treatment.

Although the surviving neurons at or near the site of injury have the ability to rearrange their anatomy and functional connectivity [2], this reconstruction can take several years, or not occur at all, due to the adverse environment produced by inflammation, ischemia, lipid peroxidation, and glial scarring, all of which limit spontaneous plasticity and decrease neuronal survival and functional recovery [3].

To protect spinal cord nervous tissue and/or to promote nervous tissue regeneration, various cells or tissues have been implanted into the injured spinal cord, including Schwann cells [4], peripheral nerves [5], fetal tissue [6], and neural stem cells [7], among others. Nevertheless, these treatments have provided only modest functional improvements because of the lack of structural and biochemical frameworks for guiding, protecting, and encouraging the regrowth of axons across the injured zone of the spinal cord. To achieve this structural support, different tissue engineering strategies have been tested that use natural and synthetic materials to create cellular or cell-free bridges through the injured spinal cord or by constructing scaffolds with various types of cells or neurotrophic factors for spinal cord repair [8–11].

Among the natural polymers used as a scaffold after a TSCI in rats are alginate, which reduces the astrocytosis and enhances axonal regeneration [12]; agarose, which supports linear axonal growth through implantation [13]; and acellular spinal cord scaffolds seeded or not with bone marrow stromal cells (BMSC), which enhance the survival of BMSC, reduce apoptosis and improve functional recovery after a hemisection or partial injury of the spinal cord [14]. The synthetic polymers used in an injured spinal cord until now have been poly(2-hidroxy-ethyl methacrylate) or poly(2-hidroxy-ethyl methacrylate)-co-methyl methacrylate (PHEMA/PHEMA-MMA), which increase angiogenesis and axonal regeneration [15, 16]; poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA), which induces the myelination of axons and reduces astrocytosis and glial scar formation [17]; and polypyrrole (PPy) polymer, which our research group has shown exhibits neuroprotective effects through increasing the amount of preserved spinal cord tissue and increases the functional recovery [18, 19]. The combined strategies include the three-dimensional nanofibrous core-sheath scaffold with a nanorough sheath and alginate core constructed on the poly(lactic-co-glycolic acid) or PGLA scaffolds, which supports axon regeneration and functional recovery after a TSCI by hemisection [20].

PPy is a non-biodegradable semiconductor polymer, which due to its conductivity and low inflammatory response has been used in vitro to promote cell adhesion, proliferation and growth of different types of cells such as neurons [21] and even to enhance neurite extension [22]. Nevertheless, the method of PPy synthesis is crucial for the construction of an adequate implant for biological systems because traditional chemical and electrochemical polymerization methods use compounds such as accelerators, solvents or intermediary products that present harmful traces to the organism and produce undesirable or adverse reactions in the body when implanted, especially if the implants are placed into the central nervous system, which has a very delicate physicochemical equilibrium. A non-contaminant method that uses electric variables instead of chemical reagents to induce the polymerization is the synthesis by plasma, which only requires the monomer (pyrrole) and dopants, without other potentially harmful compounds [23]. Our research group has used PPy iodine-doped (PPy/I) polymer synthesized by plasma for implantation into the transected spinal cord tissue, where we showed that polymer is biocompatible and is able to reduce the inflammatory response, increase tissue preservation and improve functional recovery after a lesion [18, 19]; however, the results have also shown that it is necessary to apply an additional therapeutic option to improve the functional recovery.

To date, the only acceptable therapeutic strategy used in the clinic, once the patient is stable, is passive and active physical rehabilitation with the aim of strengthening those muscles that still are active and to prevent spasticity

thereof because physical rehabilitation increases the neurotrophin levels in the muscles and spinal cord and induces and potentiates neuronal plasticity [24–26]. Moreover, active exercise performed mainly by locomotor training treadmill (TT) with or without partial body-weight support or robotic or manual assistance, has been shown to not only enhance locomotor function in both experimental and clinical studies but to also induce plasticity [27–29]. Therefore, to preserve the greatest amount of spinal cord tissue and to induce the neuroregenerative processes with the aim to obtain a higher functional recovery rate, the present study proposes the use of implants of PPy/I synthesized by plasma in therapeutic combination with TT for the treatment of a TSCI produced by contusion in rats.

2 Materials and methods

2.1 Synthesis of PPy/I implants

Thin films of PPy/I were synthesized by resistive radiofrequency (RF) glow discharges using a vacuum tubular glass reactor of 9 cm in diameter and 25 cm in length with stainless steel flanges and two stainless steel flat electrodes of 7.0 ± 0.05 cm in diameter and separated by 5.0 ± 0.05 cm. One electrode was connected to the ground and the other to the rf signal at 13.56 MHz and 80 W of an Advanced Energy RFX-600 power supply combined with a matching network. The pressure in the reactor was in the 10^{-1} mBar interval and the time of synthesis was 240 min. Under these conditions, electrical glow discharges were established with pyrrole (Aldrich, 98 %) and iodine (Aldrich, 99.8 %) used as monomer and dopant, respectively. No carrier gases or other chemical reagents were used during the synthesis. Both reactants were vaporized and entered into the reactor via separated ports and were mixed inside.

The glow discharges promoted simultaneous polymerization and doping processes producing thin films of PPy/I adhered to the internal reactor walls. After the synthesis the films were washed, swollen with acetone and distilled water, and removed from the reactor walls [23]. The films were dried and pulverized manually in an agate mortar for 10 min to obtain powder with particles of different geometry and size, called mesoparticles (MPs). Then, the MPs were sterilized in an autoclave and prepared in a suspension with saline solution at 5 mg/ml.

2.2 Characterization of PPy/I implants

The morphology of the MPs was analyzed with a Jeol JMS 5900LV scanning electron microscope (SEM) coupled with an EDS Oxford INCA-XACT probe for elemental analysis and the images were processed with the Olympus Measure IT program (Olympus Soft Imaging Solutions GMBH, Johann-Krane-Weg 39 48149, Munster, Germany). The chemical structure was analyzed by infrared spectroscopy (IR) with a Nicolette 550 spectrophotometer at 400–4000 cm^{-1} intervals using 32 scans.

2.3 Traumatic spinal cord injury

All of the animals used in the present study were maintained under standard laboratory conditions with free access to food and water and handled in accordance to the General Mexican Law in Health [30]. The protocol was approved by the Scientific and Ethics Committees of the Instituto Mexicano del Seguro Social (R-2010-3601-60).

Female Long Evans rats weighing 220–260 g were subjected to surgery under ketamine and xylazine anesthesia (77.5 and 12.5 mg/kg, respectively) intramuscular following the asepsis and antisepsis protocols. A skin incision from the middle back and the paravertebral muscles was made and the thoracic nine laminate was removed carefully, exposing the spinal cord tissue. Then, a moderate TSCI by contusion was produced using a NYU impactor and then paravertebral muscles and skin were sutured separately.

After the surgical procedures, animals were treated with an anti-inflammatory (5 mL/2L of paracetamol into the drinking water) and antibiotic (200 μL of benzatinic penicillin, in one intramuscular dose). The rats recovered from the surgery and anesthesia in an intensive care unit for small animals (Schoer Manufacturing CO., Kansas City, MO, USA). Then, they were placed into individual acrylic cages with sterile sawdust where they received food and water ad libitum. Their intestine and bladder were handled with manual expression twice a day until automatism was recovered; visual inspection was performed daily to detect any skin irritation or *decubitus* ulcers.

The rats were allocated into four experimental groups as follows: Group 1. Vehicle (30 μL of saline solution); Group 2. MPs (30 μL of MPs); Group 3. Vehicle-TT (30 μL of saline solution plus treadmill training); Group 4. MPs-TT (30 μL of PPy/I MPs plus treadmill training).

2.4 Implantation of PPy/I into the injured spinal cord zone

Two days after the TSCI, rats were re-anesthetized as described before with the aim to expose the spinal cord. Then, the rats were injected using a Hamilton syringe into the injured spinal cord zone with vehicle (saline solution) or MPs (5 mg/mL). Afterwards, animals were sutured as previously described. The analgesic and antibiotic

treatments as well as the postsurgical cares were provided similarly to that after the TSCI surgery.

2.5 Treadmill training

The animals from the corresponding groups received physical rehabilitation on a walking treadmill of 42 inches for pets which was adapted with several lanes for the experiments. The rehabilitation was applied in a quadruped position, where the animals supported 100 % of their body weight. The TT began 6 days post-injury, and lasted 20 min daily, 5 days a week for a period of 2 months. The speed of the treadmill ranged from 13.9 to 16.7 cm/s.

2.6 Functional recovery

The recovery of motor function from the hind limbs of the rats was assessed individually during 4–5 min in an open field using the Basso, Beattie and Bresnahan (BBB) scale. The test was applied by two observers blind to the treatment of each animal. The first evaluation was conducted 24 h after the TSCI with the aim to corroborate the paralysis on both of the hind limbs and then once a week during the following 9 weeks. The BBB functional scale is a 22-points test that evaluates the movements of the hip, knee and ankle joints, where 21 represents a normal function and 0 indicates no movement of none of the joints of the hind limbs [31].

2.7 Histological and morphometric analysis

Nine weeks after the TSCI, animals were anesthetized followed by an intraperitoneal administration of 0.2 mL of heparin. Then, a wide thoracotomy was performed, the ascending aorta was cannulated and 200 mL of cool physiological saline solution followed by 400 mL of 4 % paraformaldehyde in phosphate buffer were perfused through the heart, using a peristaltic pump at 30 mL/min. Then, the spinal cord was obtained including the injured zone and the neighboring areas. The specimens were embedded in paraffin. Serial longitudinal sections of 10 μm thickness were cut and stained with hematoxylin and eosin for histological and morphometric analysis. Digital images were obtained using a light microscope with a computerized system equipped with the IM 500 software and a CCD-IRIS Sony camera. The morphometric assessment was performed with the Image Database V.4.01 software from Leica. The preserved spinal cord tissue was measured in an area of 25.07 mm^2 surrounding the lesion. To have comparable evaluation areas, the ependyma was taken as a reference for the cuts.

2.8 Statistical analysis

The BBB scores were evaluated using the ANOVA of repeated measures followed by Dunnett's test. The preserved spinal cord tissue was statistically analyzed by the Kruskall–Wallis test followed by the Mann–Whitney U test. Significant differences were considered when $P < 0.05$. The analyses were performed using the SPSS 16.0 software.

3 Results

3.1 Morphology of MPs from PPy/I

Different morphological aspects of PPy/I are presented in Fig. 1, where particles of different size and irregular geometry with a tendency to form agglomerates with other neighboring particles can be observed. The MPs normal size distribution ranged from 0.006 and 25.9 μm, with a mean of 4.2 μm.

3.2 Chemical structure from MPs of PPy/I

An infrared (FT-IR) spectrum from the MPs of PPy/I is shown in Fig. 2, where the most significant absorption is found at 3430 cm^{-1}, which corresponds to the N–H bonds of pyrrole and O–H, as previously described [32]. The absorption at 2932 cm^{-1} indicates the presence of C–H aliphatic groups. The triple bonds of C≡N and C≡C are located at 2220 and 2364 cm^{-1}, and the double bonds between N, C and O such as N=C=C, O=C=C, C=C=C can also be identified in different combinations in that region. The individual double bonds, C=O, C=C, and C=N can be related with the absorption in 1630 cm^{-1}, where C=C bonds are part of the PPy structure and therefore of the polymers. The peak at 1435 cm^{-1} can be associated with the substitution of hydrogens in heteroaromatic rings. One of these possible substitutions can be identified in the small peak centered in 604 cm^{-1}, which indicates the presence of iodine in the polymeric particles.

3.3 Elemental analysis

The analysis of atomic percentage of C, N, O and I in MPs of PPy/I indicated that the C (74.78 %), N (14.38 %) and I (0.78 %) are part of MPs structure, but O (9.12 %) is an additional element that could be added by oxidation after synthesis as a consequence of the atmospheric interaction. The participation of I is low because this element has a doping function in the polymer.

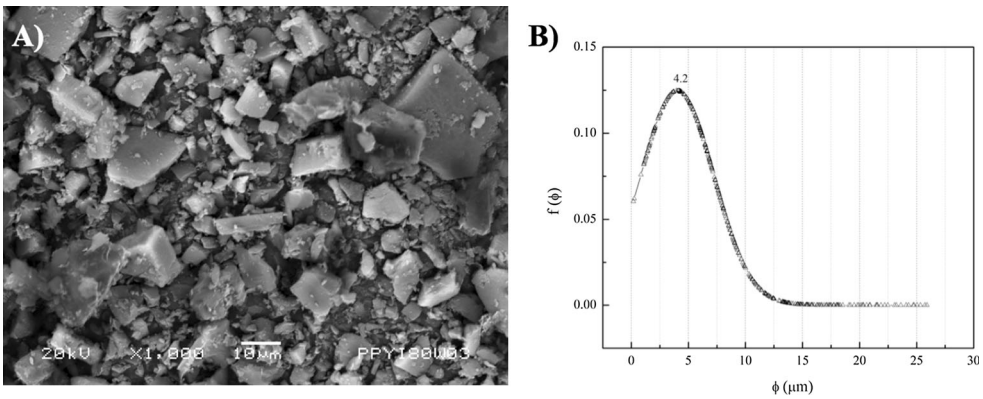


Fig. 1 **a** SEM micrographs of mesoparticles (MPs) from polypyrrole/iodine (PPy/I) obtained with a Jeol JMS 5900LV scanning electron microscope at 1000×, where agglomerated particles of different sizes and shapes can be distinguished. **b** Graphic displaying the size distribution of MPs from PPy/I

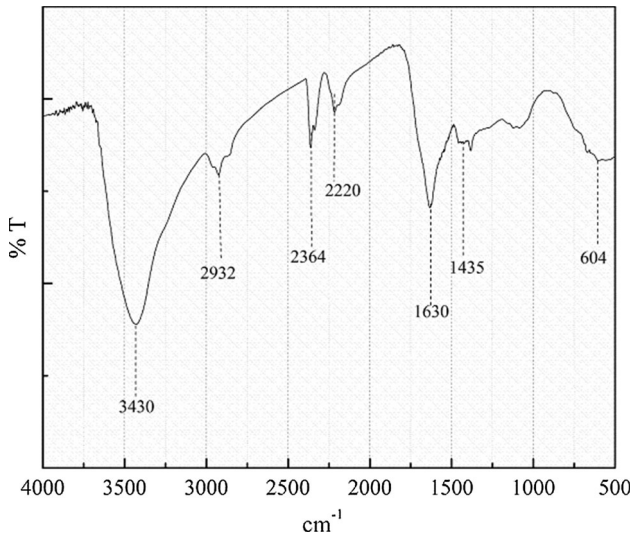


Fig. 2 Infrared (FT-IR) spectrum of mesoparticles (MPs) from polypyrrole/iodine (PPy/I), displaying the most important peaks observed during the analysis of the biomaterial before implantation into the injured spinal cord

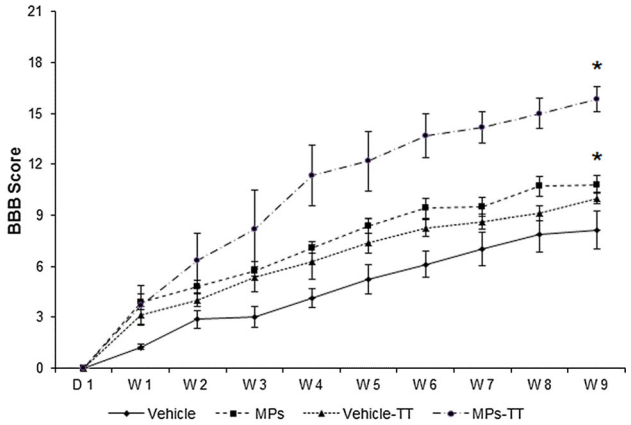


Fig. 3 Time course of functional recovery measured by BBB open field score after traumatic spinal cord injury (TSCI). Vehicle: saline solution; Vehicle-TT: saline solution plus treadmill training; MPs: implant of mesoparticles; MPs-TT: implant of mesoparticles plus treadmill training. The results are expressed as the mean ± SE. Repeated-measures ANOVA test followed by Dunnett's test. *MPs and MPs-TT groups were significantly different from the Vehicle group ($P = 0.015$ and 0.000, respectively)

3.4 Functional recovery

Motor function of the hind limbs was evaluated 24 h after the TSCI with the aim to corroborate complete bilateral paralysis in all of the rats (BBB = 0). Afterwards, the gradual improvement of each animal was assessed once a week during 9 weeks (Fig. 3). At the end of the follow up, the animals belonging to the vehicle group showed the poorest functional recovery, reaching only 8 in the BBB scale, which means that the rats had extensive movement of the three joints of the hind limbs (knee, hip and ankle) but without body weight support. Animals from the Vehicle-TT group had an average assessment of 10 in the BBB scale, which means that the rats showed extensive movement of the three joints, occasional body weight support, and plantar placement of the paw, whereas animals treated with MPs showed a better functional recovery (10.8) because they had extensive movement in the three joints of the hind limbs, plantar steps and frequent or consistent weight support (Fig. 3). The rats with the MPs-TT therapeutic combination showed the highest score (15.8) in the BBB scale at week 9, which indicated that the animals were able to consistently support their body weight and display coordination during walking between hind limbs and fore limbs, and take plantar steps with a predominantly parallel paw position to the body. This behavior was significantly different in the MPs group ($P = 0.015$) and the MPs-TT group ($P = 0.000$) from that observed in the vehicle group.

3.5 Histological and morphometric analysis

Nine weeks after the TSCI, the animals were sacrificed to analyze the preserved tissue of the spinal cord and the

implants' integration into the nervous tissue. The amount of preserved spinal cord tissue was quantified in an area of 25.07 mm^2 per each spinal cord included in the analysis. Morphometric analysis showed that a better preservation of nervous tissue was observed in the groups implanted with MPs alone ($P = 0.034$) or in combination with TT ($P = 0.05$) when compared with the group that just received the vehicle.

Photomicrographs of the spinal cords from the vehicle group showed the greatest tissue destruction, the biggest cystic cavities and a complete loss of histological architecture, with no differences between this and the vehicle-TT group ($P = 0.724$). The MPs-TT group showed a better preservation of the spinal cord tissue and fewer cystic cavities of different size at the injured zone than the rest of the groups. Although the animals with an implant of MPs displayed a good integration into the spinal cord tissue, the MPs tended to move toward the cephalic area without a response of rejection, but with an acute inflammatory response; however, this response was lower than that observed in the vehicle group (Fig. 4). Although the animals that received MPs showed a large glial scar that spread beyond the injury site toward the cephalic and caudal area, this scar was less evident when the rats received the implant of MPs in combination with TT treatment, whereas the presence of inflammatory cells was less in the implanted group that in the non-implanted groups. Moreover, no evident response of rejection to the implant of MPs was detected in any case (Fig. 5).

4 Discussion

In the present study, we evaluated the effect of MPs of PPy/I that were synthesized using plasma and implanted 2 days after a TSCI by contusion. Because others have described the functional improvement after implantation of different biopolymers to be modest (for review see [33]), one of the goal of the present work was to increase the potential beneficial effects that our research group obtained before using implantation of PPy/I [18, 19]. Thus, this therapeutic strategy was used in combination with locomotor training on a treadmill. This combination increased the functional recovery in paraplegic animals, but did not increase the protection of the nervous tissue after a TSCI. These results could be due to an acceleration in the spontaneous plasticity of the surviving neural circuits of the spinal cord, more so than by neuroprotection of the spinal cord tissue in the acute phase of the lesion because the implants were

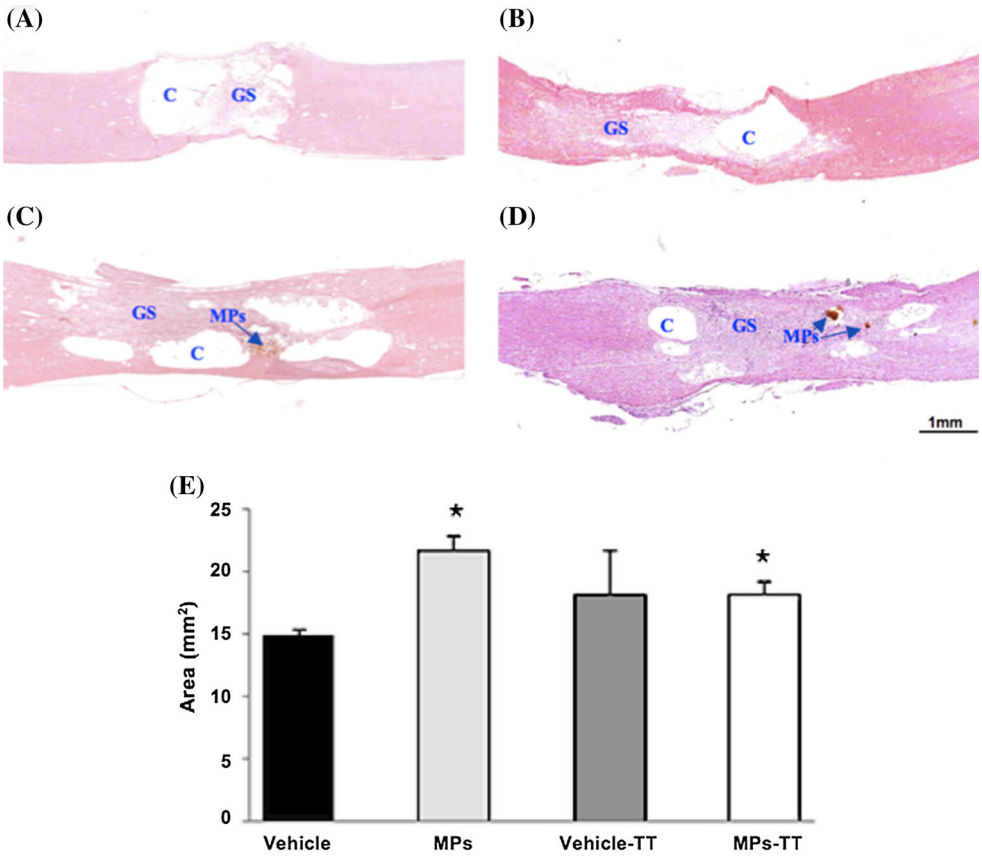


Fig. 4 Longitudinal sections of the spinal cord from representative rats from each experimental group that show the epicenter of the injured zone, the preserved tissue and the cystic cavities formed 2 months after a traumatic spinal cord injury. **a** Vehicle: saline solution; **b** Vehicle-TT: saline solution plus treadmill training; **c** MPs: implant of mesoparticles; **d** MPs-TT: implant of mesoparticles plus treadmill training. Magnification 2×. Hematoxylin/eosin stain. *MPs* implant of mesoparticles of PPy/I, *GS* glial scar; *C* cyst. **e** Graphic showing the results from the morphometric analysis where the MPs and MPs-TT groups were significantly different from the vehicle group ($P = 0.034$ and 0.05, respectively). Kruskall–Wallis test followed by Mann–Whitney test

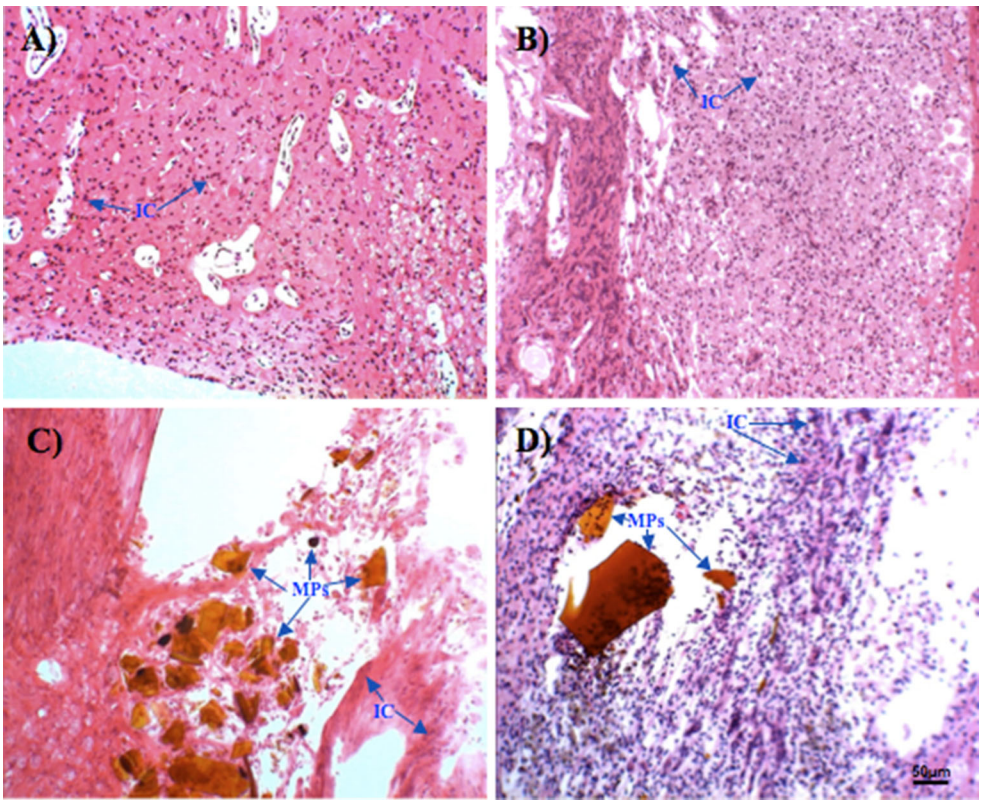


Fig. 5 Magnification of longitudinal sections of the spinal cord from representative rats of each group that show the epicenter of the injured zone, with and without the implants and the inflammatory response. **a** Vehicle: saline solution; **b** Vehicle-TT: saline solution plus treadmill training; **c** MPs: implant of mesoparticles; **d** MPs-TT: implant of mesoparticles plus treadmill training. Magnification 20×. Hematoxylin/eosin stain. *MPs* mesoparticles of polypyrrole/iodine (PPy/I) implant; *IC* inflammatory cells

injected 2 days after the TSCI and the TT began 5 days later.

In previous studies, our research group showed that implants of films of PPy/I synthesized by plasma were able to decrease the inflammatory response, produce neuroprotection and promote functional recovery when implantation is performed immediately after a complete section of the spinal cord in adult rats [18, 19]. Nevertheless, in humans, the TSCI is usually produced by contusion and not by complete section, where surgical manipulation of the nervous tissue by implantation of scaffolds, films or solid neuronal bridges can produce further damage. So, in the present study, the TSCI model by contusion was used and the biomaterial was applied as MPs into the injured zone by direct injection 2 days after the lesion. Under these conditions, after 2 months, the MPs promoted more tissue preservation and functional recovery in comparison with the animals that received just the vehicle; however, the functional results improve if both therapeutic strategies were used together although without an increase in the amount of preserved tissue. So, this combination could stimulate specific mechanisms of neuronal plasticity in the preserved spinal cord tissue more that stimulate mechanisms of neuroprotection or neuroregeneration.

Although different injectable biomaterials have been used previously in rats as implants after a TSCI such as collagen, fibrin, fibronectin and poly (lactic acid)-polyethylene glycol-poly(lactic acid), those implants enhance and support the oriented growth of axons but with a modest functional recovery [33–35]. Cigognini et al. [36] reported a self-assembling of the peptide RADA 16-I functionalized with a bone marrow homing motif (BMHP1) and optimized via the insertion of a 4-glycine-spacer (RADA16-4G-BMHP1) that was implanted immediately after a TSCI by contusion in rats. This peptide provided both physical and trophic support to nervous tissue ingrowth, increased cellular and axonal infiltration within the cyst, and improved functional recovery. Other researchers studied the effects of collagen, viscous fibronectin (FN), fibrin (FB) and fibrin/fibronectin (FB/FN) injected into an experimental cavity in the spinal cord of rats. A number of them displayed good integration with the host and were able to support axonal ingrowth in association with the infiltration of Schwann cells and deposit of laminin [37]; however, the

collagen implant resulted in uneven axonal growth because these implants contained a dense inclusion that the axons were not able to penetrate, whereas the animals implanted with FN had large cavities at the interface between the implant and host, and few surviving neurons in the intact spinal cord adjoining the implant site. Only the animals implanted with FB/FN showed a robust growth of axons [33]. Among the main characteristics of these materials is their rapid transformation from a liquid to a gel after being injected into the injured spinal cord and the formation of a scaffold thereafter; however, even if the implants show good integration into the nervous tissue and they promote axonal regeneration, one must consider that they are biodegradable materials. Thus, it is important to take note of any degradation products that can be produced because they could be toxic for the host, as well as the degradation rate, which should be synchronized with the frequency of nerve regeneration with the aim to avoid the risk of nerve compression or limitation of axonal growth. In the present work, the MPs did not generate a scaffold because they were suspended into a saline solution (vehicle). Thus, the MPs were dispersed throughout the lesion site and the surrounding areas without the risk of compression and cell growth between the implant was not limited. Although the MPs of PPy/I are non-biodegradable, the risk of toxicity was reduced because they were synthesized by plasma. Moreover, the implantation of MPs into the injured spinal cord was well-tolerated as it generated a discrete acute inflammatory response with no response of rejection, and no additional alterations in the animals' health during the 2 months of follow up. This positive effect could be also due to the method through which the implants were synthesized because if the MPs of PPy/I are obtained by plasma, no potentially toxic external agent is included during polymerization as is the case for traditional chemical and electrochemical methods [23]. In the polymerization by plasma, the reactions are performed by means of electric discharges that ionize the reactant monomers on their gaseous phase and the produced plasmas. Molecules, electrons and ions increase their kinetic energy and collide, breaking some chemical bonds in the monomers that will form the polymer, where only chemical compounds involved in this process are the monomers and its derivatives. This mechanism partly explains why polymers synthesized by plasma have different physical and chemical properties than those obtained by traditional chemical or electrochemical methods.

At the end of the follow up, the animals implanted with MPs of PPy showed a significant functional recovery in comparison with the animals that received just the vehicle, most likely due to the implant's physical and chemical properties. Polymer's conductivity is able to alter the local electric fields and modify the extracellular matrix and the environment around the implant, promoting neuronal interactions, preventing cell death, controlling cell growth and inducing plasticity [38–41]. Because the resulting polymer synthesized by plasma can be a three-dimensional cross linked structure, this structure may affect the conductive properties of PPy. Moreover, the polymer was combined with iodine (PPy/I) during the synthesis with the aim to increase its ability to transfer electrical charges and thus negate a requirement for external sources of electrical stimulation for exerting its effect. Furthermore, the heteroaromatic amine groups of the polymer are well-tolerated by cells and they can promote interactions between them; however, more studies are needed to understand which molecules and neurotrophic factors are specifically involved in this process, and which mechanisms are responsible for improving functional recovery in the animals that received the implant of MPs of PPy/I.

Even though the implant of MPs had positive effects on neuroprotective processes and functional recovery after a TSCI, the best functional results were obtained when the implant of MPs was used together with the therapeutic strategy of rehabilitation with TT. Although different strategies of rehabilitation, such as swimming, bicycling, exercise via wheel running and TT have been applied in experimental models of TSCI with the aim to improve locomotion, not all of them have had a significant effect on functional recovery. Some authors showed no improvement in functional recovery assessed with the BBB scale using swimming as rehabilitation therapy, and compared with untrained animals with a TSCI by contusion since it has been proved that swimming induces extravasation in and around the site of injury [42]. Bicycling has been shown to help prevent muscle atrophy and restore the frequency-dependent depression of the H-reflex to the level of that of intact animals but without significant effects on the functional recovery in rats with spinal cord transection [43, 44]. Although exercise via wheel running has improved functional recovery in rats with contusion [45], TT with or without partial body-weight support or with robotic or manual assistance after a TSCI has a better effect on functional recovery because it engages new coordinative strategies that, although different from normal locomotion, generate foot motion very similar to normal; promotes reorganization of locomotor networks, both above and below the lesion site; and induces rostral and caudal plasticity to the injury zone because the circuits that control stepping as well as standing are highly plastic and their functional connectivity can be modulated by TT [46, 47]. Others have shown that TT induces plasticity by enhancing axonal outgrowth and Erk1/2 activation and promotes functional recovery in rodents and cats with spinal cord section or contusion [48–50]. In the present work, TT was implemented without body weight support,

with the aim that rats were able to walk voluntarily in their natural quadruped position, and thus the confusing variables associated with movements without voluntary control in a bipedal position due to external manipulation of researcher were avoided.

Although it was previously shown that TT promotes functional recovery, any protection of the nervous tissue was demonstrated in comparison with untrained animals in the present work. Nevertheless, results suggest that TT could induce and accelerate the spontaneous plasticity in the surviving axons and spinal cord circuits by altering their organization, structure and function, more so than by forming new fibers, which gives rise to better functional recovery when used in combination with PPy/I implants, but in a non significant way when applied alone; however, in accordance with other reports using rats with moderate contusion and TT without body weight support, TT is able to improve locomotor function through increasing the neuroplasticity in the spinal cord neural circuits where the recovery directly depends on TT rather than the spontaneous reorganization of the spinal circuits [51]. Therefore, untrained animals achieve an incomplete degree of recovery due to the spontaneous plasticity, which is limited.

In the present work, the combined therapeutic strategy of the implant of MPs plus TT had an additive effect on functional recovery. In the animals from the group that received the implant of MPs, a better functional recovery was observed than in the animals from the groups without the implant, from the first week post injury to the end of the follow up. Nevertheless, when the implant of MPs was combined with TT, the animals exhibited faster recovery than the animals in the other experimental groups until the fourth week. Thereafter, recovery continuously increased, although at a lower rate; however, no additive effects were demonstrated when both treatments were applied together. The results suggest the possibility that the implants of MPs alone induce plasticity due to the reorganization of the surviving neural circuits. When the implants are combined with TT, this plasticity is accelerated even more, but without direct association with the amount of preserved tissue, thus partly explaining why the combination of both therapeutic strategies produces better functional results than if they are applied separately.

Despite previous work, it is important to continue studying the physicochemical and morphological properties of the biomaterial and test different vehicles that prevent the mobilization of MPs far away from the injury site to improve the beneficial responses, in addition to studying their effect in the chronic phase. Furthermore, if implants of MPs of PPy have future applications in biomedicine, mainly in the central nervous system because of their size and the possibility to apply them without further damage to the nervous tissue, the use of injectable biomaterials for the treatment of TSCI is still being tested; thus, it is necessary to continue studying and developing new strategies that may enable this treatment to be applied to humans with TSCI alone or in combination with other therapies to further enhance the previous and present results.

5 Conclusions

The implants of MPs of PPy/I synthesized by plasma displayed good integration into the nervous tissue without a rejection response when they were applied 2 days after a TSCI.

Although no additive effect on the preservation of nervous tissue was demonstrated when the implantation of MPs of PPy/I was used in combination with TT, the promotion of functional recovery was positively affected.

The use of both therapeutic strategies by separately could stimulate mechanisms of neuroprotection and neuroregeneration, but when using together they could potentiate the stimulation of mechanisms of neuronal plasticity in the preserved spinal cord tissue after a TSCI and produce a significant functional recovery.

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Compliance with Ethical Standards

Conflict of interest No potential conflict of interest relevant to this article is reported.

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