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# Pyrrole Plasma Polymer-Coated Fibrillar Scaffold Implant: Pilot Study in Rat Spinal Cord Transection with MRI

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*Abstract*— **Despite extensive research on spinal cord injury (SCI) therapies for the recovery of motor, sensory and autonomic function, currently there are no effective treatments to completely restore tissue structure and function. In this work, a polylactic acid (PLA) fibrillar scaffold coated with pyrrole plasma polymer doped with iodine (pPPy/I), was studied as therapeutic strategy in a SCI transection model. Magnetic resonance imaging (MRI) was used to evaluate tissue response to the implant. Behavioral analysis using the BBB open-field testing was conducted to evaluate functional response. MRI analysis showed the SCI model completely disrupted tissue continuity, and diffusion indices were altered at the injury site. The animals had completely paralyzed hindlimbs and bladder control loss after injury. After 8 weeks of treatment, in contrast to control and PLA-implanted animals, PLA+pPPy/I-implanted animal had regained bladder control autonomy and frequent to consistent weight supported plantar steps and occasional coordination between forelimbs and hindlimbs. These results suggest fibrillar scaffolds coated with pPPy/I constitute a promising therapy for SCI.**

## I. Introduction

Spinal cord injury (SCI) severely affects motor, sensory and autonomic function, and is associated to the disconnection of neural circuits and failure of neural tissue regeneration. After a SCI, complex processes take place at the injury epicenter, including hemorrhage, inflammation, ischemia and reactive glia migration, resulting in the formation of a glial scar at chronic stages [1]. Recent studies have demonstrated that a combinatorial strategy including biomaterials, stem cells, rehabilitation and stimulation, improves functional and histoarchitectural outcomes [2].

Neural tissue engineering as a strategy for nervous tissue reconstitution and ultimately functional recovery, comprises the use of biocompatible materials designed to structurally mimic the nervous tissue environment to promote cell adhesion, migration, differentiation, and survival [3]. Fibrillar scaffolds constitute a platform with a porous microstructure that promotes cell and nutrient migration, fabricated with biomaterials which facilitate cell-surface interactions to promote tissue-sparing and regeneration [4].

Plasma synthesized polypyrrole doped with iodine (pPPy/I) is a biocompatible material which has been studied as an implant, in the form of particles in suspension in contusion SCI models, and as tablets in transection models [5]–[9]. These studies demonstrated the pPPy/I implant results in motor recovery and neuroprotection of the spinal cord at the injury site, reducing inflammation and lesion effects, and maintaining histoarchitecture. Although contusion may better model the most common injuries documented at the clinic level, transection models are eligible in regeneration studies since this model completely disrupts tissue continuity and differences between spared and neo-tissue can be clearly stated [10].

In this work, polylactic acid (PLA) was electrospun to fabricate porous fibrillar scaffolds which were coated with pPPy/I (PLA+pPPy/I) as a combined treatment strategy in a SCI transection model, adding an architectural component to pPPy/I.

## II. Materials and Methods

### A. Implant fabrication

Scaffolds were prepared by electrospinning a 15% v/v solution of PLA (Ingeo 3251D) in chloroform and dimethylformamide (9:1), using a 20kV electric field between a 0.6 mm internal diameter stainless-steel needle and a grounded collector plate. The distance between the plate and the needle was adjusted to 22 cm. PLA solution was pumped at a 2ml/h flow rate through the needle. The scaffold was then maintained in a vacuum oven for 4 days for residual solvent evaporation.

Implants were prepared by cutting a 4x3 mm section of scaffold, folded twice, and coated with pyrrole plasma polymer doped with iodine (pPPy/I) synthesized in a glass reactor as described previously [11]. Briefly, the PLA implants were fixed to the reactor between stainless steel electrodes connected to a 13.5 MHz RF generator, placed 10 cm apart. The plasma polymer synthesis was conducted for 30 minutes at 1±0.1 Torr, 30W. Resulting implant constructs are shown in Fig. 1.

### B. Surgical procedure

PLA and PLA+pPPy/I implants were sterilized under UV radiation for 30 minutes before implantation. Animal handling procedures were consistent with national and international regulations. Adult female Wistar rats (N=3) were anesthetized with a 0.1ml IM injection of xylazine before a 0.04 ml IP injection of Zoletil 50. The spinal cord was transected with micro scissors after laminectomy at T9 level and a longitudinal incision of the meninges. Implants were adjusted to 3x3mm sections and placed between the severed segments of the spinal cord. The rat used as control received no implant. A 5x3mm patch of a single sheet of electrospun PLA coated with pPPy/I was placed over the incision of the meninges of all the animals.

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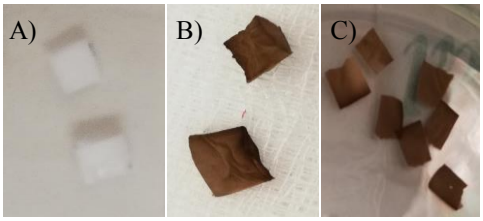


Figure 1. Fabricated costructs. For surgical implantation, the constructs were adjusted to a 3x3x1mm rectangular shape implants. A) PLA implants, B) PLA+pPPy/I implants, C) PLA+pPPy/I patches for meninges incision repair.

Finally, muscles and skin were sutured in layers, and the animals received postoperative care.

### *C. Implant assessment*

The 2nd and 4th week of treatment, structural development of the injury site was evaluated with Magnetic Resonance Imaging (MRI), using a 3T Philips Achieva MR system, coupled to a neurovascular array coil. T1-weighted, T2-weighted, and diffusion tensor (DTI) sequences were performed and analyzed in Weasis Medical Viewer 3.6.2 to assess structural changes in neural tissue.

DTI and tractography analysis were conducted in DSI Studio [12]. A total of 32 diffusion sampling directions were acquired to calculate the DTI, with a b-value of 800 s/mm$^2$. Resulting in-plane resolution was 1.4 mm and slice thickness was 1.5 mm. The diffusion tensor was calculated and a deterministic fiber tracking algorithm was used to estimate axon fibers trajectories along regions placed over the spinal cord [12]. Using thoracic intact fibers (craniocaudal blue-color coded) as reference, the injury site was identified and 3 adjacent regions in rostral and caudal direction studied for tract-tracing. The anisotropy and angular thresholds were 0.2. and 20 degrees, respectively. The step size was 0.2 mm. The fiber trajectories were smoothed by averaging the propagation direction with 50% of the previous direction. Tracks with length shorter than 1 or longer than 300 mm were discarded. A total of 5000 tracts were calculated.

Motor recovery was assessed with the BBB rating scale in an open-field environment before injury and weekly up to 8 weeks of treatment [13]. A total of 4 minutes of free movement were recorded and analyzed each session.

## III. Results and Discussion

MRI on the 2nd week post-surgery showed SCI procedure resulted in the complete transection of the tissue, and a visible gap of 0.6 - 0.9 mm between stumps, as shown in Fig. 2. In contrast to contusion SCI models, in which possible remaining tracts may survive primary damage, transection models eliminate any connection between the brain and the spinal cord below injury site, thus preventing transmission of any bioelectrical and biochemical signals, leading to motor and sensory loss [14].

Fractional anisotropy (FA), mean diffusivity (MD) and tract-tracing (tractography) from regions adjacent to and at injury site of the of the control, PLA- and PLA+pPPy/I-

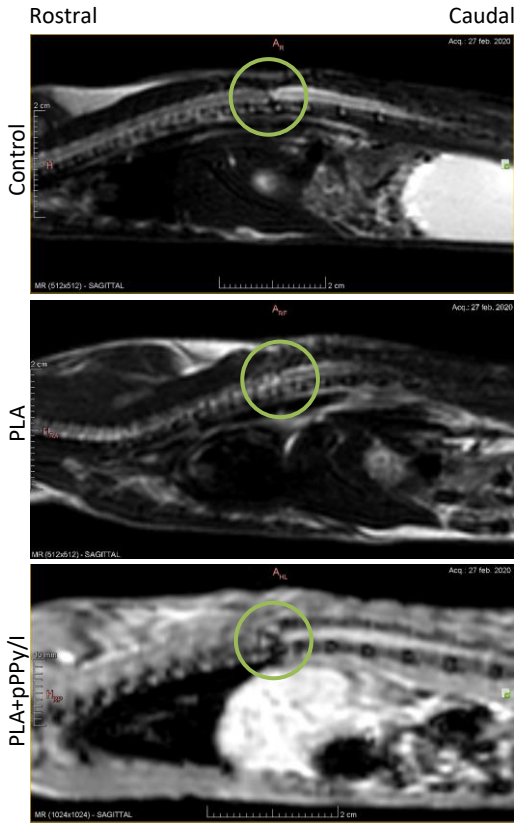


Figure 2. MRI analysis on the 2nd week. T2W (control and PLA) and T1W (PLA+pPPy/I) sagittal view images showing the injury site.

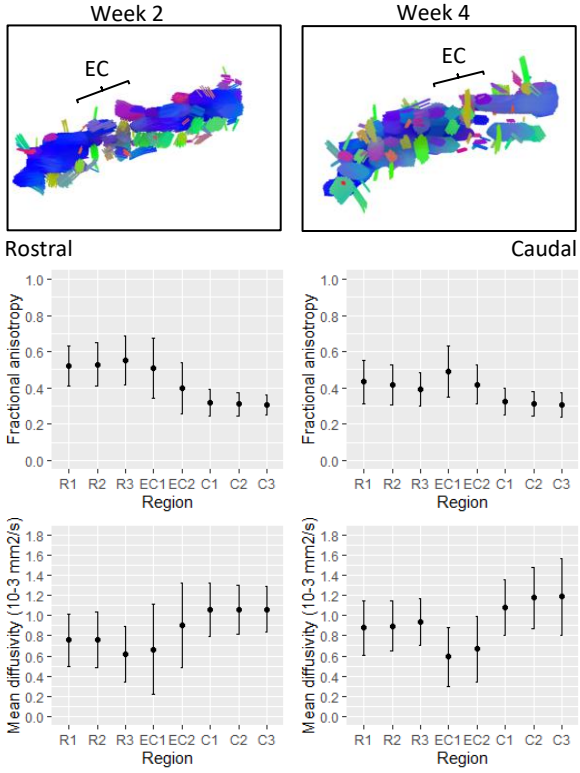


Figure 3. Tractography and DTI indices FA and MD of control animal at week 2 and 4 (mean±SD). C = caudal direction, EC = epicenter of the lesion, R = rostral direction.

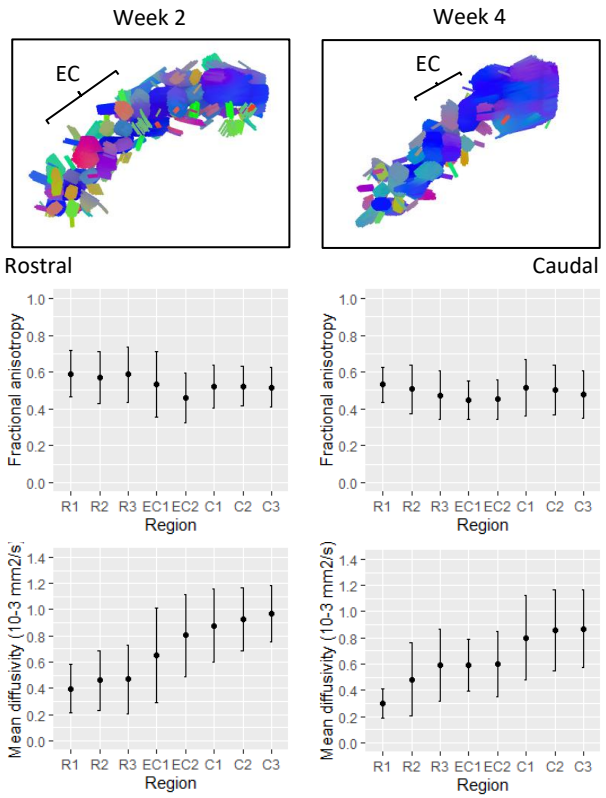


Figure 4. Tractography and DTI indices FA and MD of PLA-implanted animal at week 2 and 4 (mean±SD). C = caudal direction, EC = epicenter of the lesion, R = rostral direction.

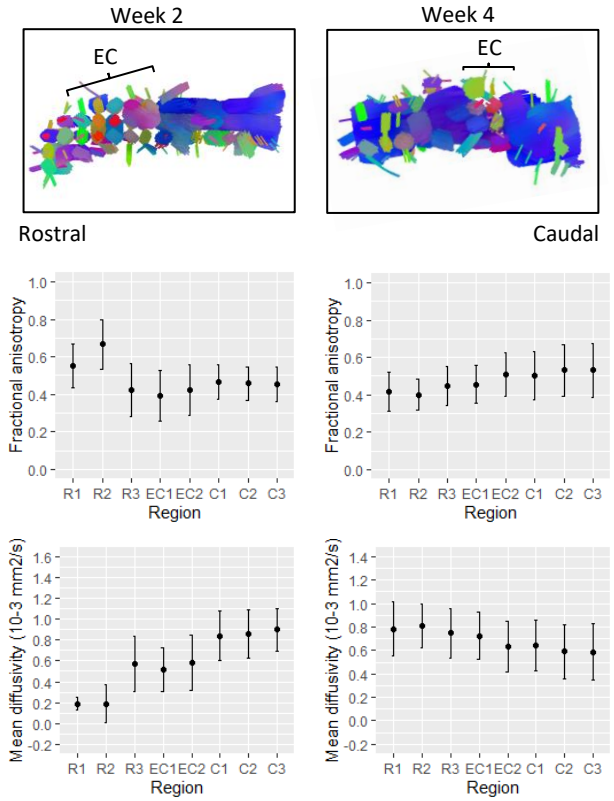


Figure 5. Tractography and DTI indices FA and MD of PLA+pPPy/I-implanted animal at week 2 and 4 (mean±SD). C = caudal direction, EC = epicenter of the lesion, R = rostral direction.

implanted animals are shown in Figures 3, 4 and 5, respectively. FA reflects structure directionality of the tissue, FA=0 isotropic diffusion and FA=1, anisotropic diffusion. MD (equivalent to apparent diffusion coefficient, ADC), reflects the average diffusivity. High MD values reflect isotropic diffusivity, which is associated to inflammation, cysts, and loss of fiber structure in general [14].

Since effective signal transmission through the spinal cord from the brain to peripheral nerves is associated to integrity of white matter tracts and myelinated fibers, spinal cord should display high FA values and low MD values [14].

As shown in Fig. 3, low values of FA and high MD values suggest inflammation, hemorrhage, or cerebrospinal fluid (CSF) leak at the caudal side of the injury site due to the transection procedure [15]. These values have an opposite trend at the rostral side, due to the tissue being mainly anisotropic toward cervical regions. At the 4$^{th}$ week, FA values at the rostral side show a decrease with respect to the 2$^{nd}$ week, suggesting retrograde tissue loss, possibly necrosis and cyst formation at and adjacent to the injury site. These results are in accordance with MD values, tractography and T2W images, where hyperintense caudal regions are related to FA and MD values.

In contrast, Figures 4 and 5 show that PLA- and PLA+pPPy/I-implanted animals presented lower levels of inflammation, but a clear loss of structure due to transection and implant, which casts no diffusion signal. No significant changes in FA or MD values were identified from week 2 to week 4.

Locomotion test results are shown in Fig. 6. After injury, all animals displayed a complete lack of movement of the hindlimbs, and bladder dysfunction. Throughout the 8 weeks of study, control and PLA-implanted animals showed minimal joint movement, whereas the PLA+pPPy/I-implanted regained autonomous bladder control after the second week of treatment, accompanied by a score of 7 (extensive movement of hips, knees, and ankles). By the 8th

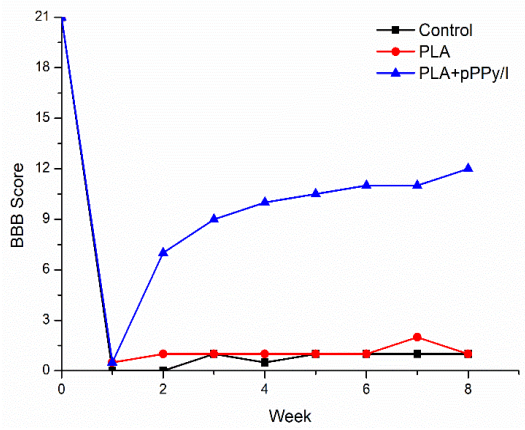


Figure 6. BBB open-field locomotion test results. Control and PLA-implanted animals achieved movement of one or two joints (hip and knee). PLA-implanted rat showed slight movement of the ankles at the 7th week. PLA+pPPy/I-implanted rat achieved a score of 7 at the 2$^{nd}$ week (extensive movement of hips, knees, and ankles). By 8th week it scored 12 (frequent to consistent weight supported plantar steps and occasional coordination between forelimbs and hindlimbs).

week it scored 12 (frequent to consistent weight supported plantar steps and occasional coordination between forelimbs and hindlimbs).

MRI anatomical images at the 4th week, are shown in Fig. 7, where control and PLA-implanted animals still display a clear gap between stumps and a slight reduction in tissue diameter at the lesion epicenter. In contrast, PLA+pPPy/I-implanted animal shows no retraction at the injury epicenter, suggesting that the implant provided neuroprotection and prevented further tissue damage, by the use of pPPy/I. In addition, two possibilities may further explain the functional recovery of the PLA+pPPy/I-implanted animal: a) the implant provided a bridge between the spinal cord stumps, which may be promoting connection through the injury site, facilitated by the pPPy/I coating; b) regenerating axons supported by the implant may be present but are not yet visible with the imaging techniques used in this work.

In any case, further evaluation of the tissue at the injury site is required, providing higher resolution of histoarchitecture, along with more experiments to draw conclusions. In addition to MRI studies, these experiments should include histology of the injury site and the use of biomarkers to search for spared tissue, neurite elongation through the scaffold, glial cell infiltration and colocalization with neural tissue [5], [16].

## IV. Conclusion

A fibrillar scaffold coated with pPPy/I was implanted as a treatment strategy for a spinal cord transection injury model. Although it is necessary for more experiments and for a larger number of animal subjects, preliminary results presented in this paper show that the implant design and materials are a promising treatment strategy for the spinal cord injury as severe as the transection model. These preliminary results show that the PLA+pPPy/I implant promoted a notable improvement of the motor function and the recovery of autonomous bladder control after the second week.

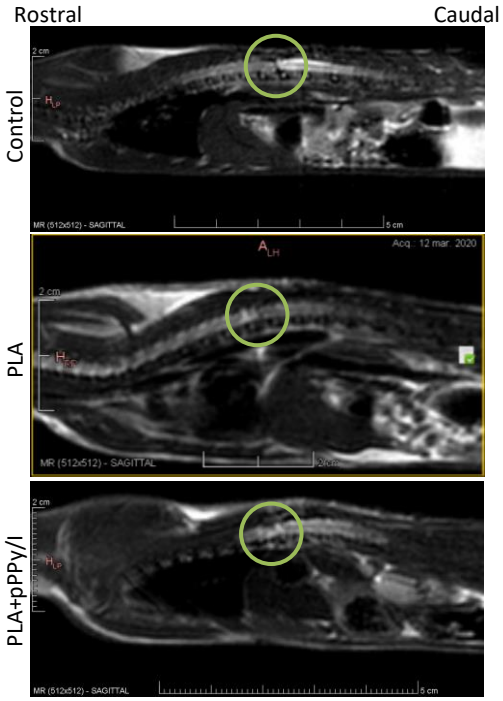


Figure 7. T2W images at week 4, showing the injury site with persistent disconnection of the spinal cord stumps in control and PLA-implanted animals. PLA+pPPy/I-implanted animal showing no tissue retraction at the injury site.


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