Contribuciones del Instituto Nacional de Investigaciones Nucleares al avance de la Ciencia y la Tecnología en México

Edición conmemorativa 2010

Chapter 1

Strategy for the Treatment of Spinal Cord Injuries after Complete Section Using Polymeric Implants Synthesized by Plasma

English version of:

Capítulo 1

Estrategia para el Tratamiento de Lesiones por Sección Completa de Médula Espinal Basada en Implantes de Polímeros Sintetizados por Plasma

Published originally in the book:

Contribuciones del Instituto Nacional de Investigaciones Nucleares al Avance de la Ciencia y la Tecnología en México
Edición Conmemorativa 2010

Translation: Guillermo J. Cruz Cruz

Chapter 1

STRATEGY FOR THE TREATMENT OF SPINAL CORD INJURIES AFTER COMPLETE SECTION USING POLYMERIC IMPLANTS SYNTHESIZED BY PLASMA

Guillermo J. Cruz Cruz, Ma. Guadalupe Olayo González
Departamento de Física, Instituto Nacional de Investigaciones Nucleares

Hermelinda Salgado Ceballos
Unidad de Investigación Médica en Enfermedades Neurológicas, Hospital de Especialidades, Centro Médico Nacional Siglo XXI del Instituto Mexicano del Seguro Social y Proyecto Camina A.C.

Araceli Díaz Ruiz, Luis Camilo Ríos Castañeda
Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía

Roberto Olayo González, Juan Morales Corona
Departamento de Física, Universidad Autónoma Metropolitana Iztapalapa

Ana Laura Álvarez Mejía, Rodrigo Mondragón Lozano, Axayácatl Morales Guadarrama
Departamento de Ingeniería Eléctrica, Universidad Autónoma Metropolitana Iztapalapa

guillermo.cruz@inin.gob.mx

1. Introduction

Spinal cord injuries in people are mostly the result of hits, compressions and tears at the spinal cord tissue that produce extensive damage to sensitive, motor and autonomic functions (temperature, visceral control, etc.). The origin of these lesions is mainly due to car accidents, firearm lesions, sports, falls or accidents at work.

Since the brain communicates with the rest of the body through the spinal cord, its lesions cause the interruption of such communication, from the segment of lesion to the lower portion of the body, causing dysfunctions and paralysis of diverse magnitudes in the disconnected extremities and organs. All nerves located in the upper section continue functioning normally. Therefore, the closer the lesion is to the brain, the greater percentage of the body will be paralyzed.

Each spinal cord injury is different and depends on the origin, mechanism of lesion, level of spinal cord affected, severity of damage and on the response of patients to this process. There are partial lesions which diminish sensations but allow some motor functions, others allow for some sensation but reduce movement, and there are also complete lesions which suppress sensations and movement alike. The international scientific community has made great efforts in this area to minimize the devastating consequences of this pathology. Nevertheless, there is currently no successful medical strategy to revert the effects of spinal cord injuries.

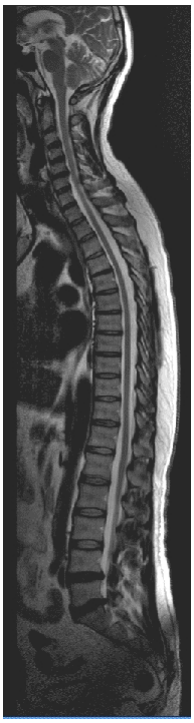
To globally understand the problem of spinal cord injuries and the treatment strategy presented in this work, the most important parts of biological systems and biomaterials related to the spinal cord are described in the following sections.

2. Central nervous system

The central nervous system of the human being is composed by the spinal cord and the encephalon (figure 1) and both are firmly protected by the bones of the vertebral column and the cranium, respectively. The spinal cord is a tissue shaped like a cylinder located inside the vertebral column while the encephalon is a semispherical tissue located in the upper section of the spinal cord, inside the cranium. The encephalon is responsible for processing information and coordinates all functions of the organism. However, as it is isolated, protected and confined within the cranium, it requires a mode of communication with the rest of the body; which is what the spinal cord is responsible for. During our first years of life, the spinal cord grows with a length proportional to the vertebral column, but with time, the vertebral column exceeds the length of the spinal cord; so that once reaching adult age, the length of the spinal cord is approximately 70% that of the vertebral column, going from the encephalon to the 1st or 2nd lumbar vertebrae.

The spinal cord is composed of various sets and subsets of tissue which transport signals between brain and body through chemical-ionic pulses. The signals are messages related to the functioning of organs, bones and muscles or sensations such as pain, cold and heat.

The center of the spinal cord contains groups of butterfly shaped neuronal bodies called gray matter. On the dorsal part of the structure, the sensitive neurons are found; on the ventral part, the motor neurons; and around that are the nerve fibers called axons which combined make up the white matter. At the center of the spinal cord a cylindrical structure is found named ependimary canal through which cerebrospinal fluid circulates. The whole spinal cord is covered by three layers of tissue called meninges: duramater, arachnoids and piamater.


(a)


(b)

Figure 1. (a) MRI showing the central nervous system in a human being. (b) Lateral X-ray image of a traumatic lesion in the spinal cord by fracture and displacement of the vertebral column.

3. Vertebral column

The vertebral column is a set of twenty four bones called vertebras which adequately aligned are the main support of the body. It is a bone structure in which three curvatures are visible. The superior one is found in the cervical zone which consists of seven small vertebras. In the middle curvature, the thoracic zone is found made up of 12 medium vertebras. In the third curvature, the lumbar zone is found which has the five larger vertebras. There also exist 2 additional bones in the lower extreme called sacrum and coccyx.

The vertebras are formed by a central body and a lateral ring called the spinal canal which contains the spinal cord. There are ramifications from the spinal cord called nervous roots emerging through 2 lateral openings located in between adjacent vertebras connecting the spinal cord with the different organs and tissues of the body.

Between the central body of each vertebra, a soft tissue is found in the shape of a disc whose function is to reduce friction among the bones, avoiding intervertebral contact by absorbing the forces generated between them. The intervertebral discs are made up of two parts, an elastic pulpy nucleus which deforms when a vertebra pushes against another and an external fibrous ring which is much stronger than the nucleus. The spinal column keeps each one of these vertebras in position using muscles, ligaments and tendons.

This whole complex system is designed so that the spinal cord can be housed in the space within the vertebras with the possibility of communicating with the different organs in the body without suffering damage due to the constant movement of the vertebral column. If one of the vertebras were to fall out of place exceeding the tolerance of normal movements, it would apply pressure on the spinal cord causing damage.

The wear and tear or excessive pressure on the intervertebral discs can also cause the discs to slide towards the spinal canal forming a hernia which puts pressure on the nervous roots producing pain, numbness, or weakening at the affected area. This type of hernia can occur in any part of the vertebral column but it is most frequent in the cervical and lumbar zones. Although these types of lesions are very painful and can have severe consequences, they are not lesions directly associated with the spinal cord since they affect mainly the peripheral and not the central nervous system. This is very important since each system has different characteristics.

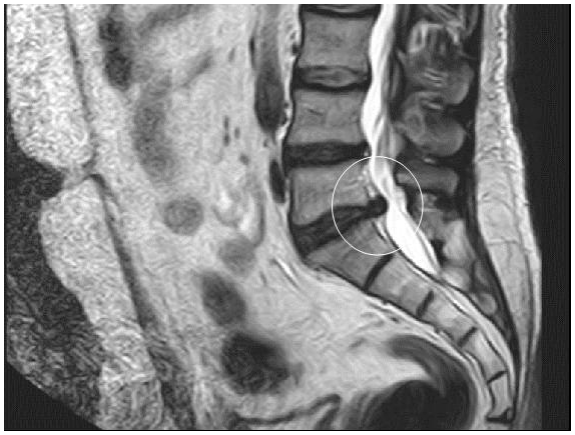


Figure 2. MRI of a hernia in a lumbar disc.

4. Neurons

The basic anatomical and functional unit of the nervous system is a highly specialized cell called neuron. This cell consists of a central body surrounded by clusters of small thin terminals, dendrites, from which small structures, spines, and an arm extension, axon, emerge. The transmission of information from one neuron to another is a complex phenomenon called synapses in the form of chemical-ionic pulses which develop because of the specialized contact with different parts of a neuron to another. The voltage levels of these ionic pulses are just a few tenths of milivolts.

Synapses depend on chemicals called neurotransmitters to develop communication, which occurs in complex combinations between dendrites, spines and axons. Because all the synapses have to be functional, the extensions of astrocytes, a kind of glial cell, occupy the space where there should be no communication between one neuron and another, avoiding interneuronal contact.

The anatomical and functional complexity of the central nervous system is due to its great number of neurons. In the adult brain, it ranges from 30 to 100 billion of neurons, which can form up to 10^{18} connections.

Once the body created neurons in the early stages of life, their number hardly increases since the cells are not able to reproduce under normal conditions. Therefore, any injury involving neuronal death reduces definitively the number of neurons producing irreversible damage. The situation becomes even more complicated because the neuronal death itself produces many reactions in the surrounding area, which weaken and kill many of the healthy neighboring neurons. Thus, an injury that involves a small neuronal damage can be magnified by several orders of magnitude through a secondary damage to neighboring neurons. Preventing secondary neuronal damage is one of the most studied therapeutic strategies to reduce the negative consequences of spinal cord injuries [1].

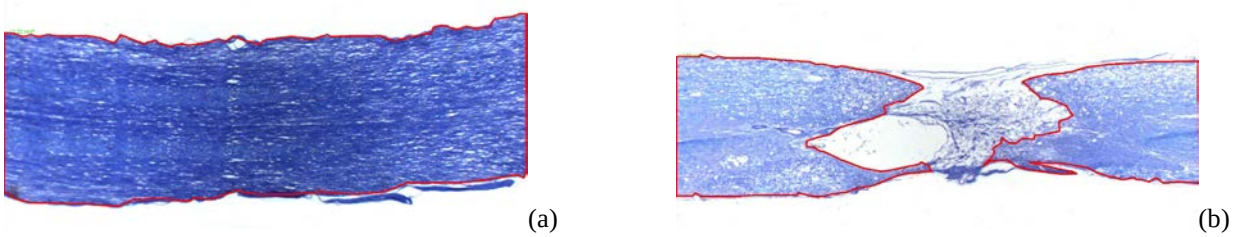
The human body has the necessary neurons to perform all bodily functions and a surplus sufficient to replace them several times. So that, once the body controls the secondary damage following an injury, the functions of lost neurons can be replaced by other healthy neurons, until their number is insufficient or their activity is saturated. This does not mean there are neurons patiently waiting to replace the injured ones, but all are active, with the potential to produce new synapses if necessary.

5. Spinal cord injury

When the spinal cord is cut as a result of a traumatic injury, several mechanisms are triggered to control and repair the damage. The neurons that survived the injury try to communicate with those who survived beyond the site of injury and *vice versa*, but if in a certain amount of time they are not successful, a healing process begins by insulating the two damaged ends. Additionally, some cysts with fluid are formed in the injury zone that complicates even more the reconnection of the spinal cord (Figure 3).

Since inception into the womb, the brain and spinal cord grow with little contact with the rest of the body, because they are semi-isolated by the bones of the skull and vertebral column, and by barriers formed by blood vessels and nerve tissues which select the passage of chemicals and immune system cells.

Therefore, the body's defense systems against foreign aggression only partially recognize the central nervous system. This seems to be the reason that, if an injury breaks the barrier between the central nervous system and the rest of the body, the antibodies confuse the latter as an invader; attack it and try to isolate it. This process is part of the previously described neuronal damage and is responsible for worsening spinal cord injuries [2].



(a) (b)

Figure 3. Images that demonstrate irreversible damage to the spinal cord at different times a) longitudinal cut of the spinal column on a normal rat, b) longitudinal cut on the spinal cord of a rat two months after the lesion where the evolution of the destruction of the spinal cord is shown, the formation of a glial scar and cysts in the area where viable tissue no longer exists. Histological technique luxol fast blue.

6. Treatment of spinal cord injuries

As noted previously, there are different mechanisms that can injure the spinal cord, such as strokes, compression, tears and cuts of the nervous tissue. This work focuses on the problem of injury by complete transection of the spinal cord, which is one of the most severe cases of spinal cord injuries. In this type of injury many strategies are experimentally studied for immediate implementation in order to protect healthy tissue and inhibit or reduce secondary damage. These strategies are generically referred to as neuroprotection.

The next step in the treatment of these lesions is based mainly on recovery of lost functions, implanting cells or biomaterials in the area of lesion to aid in neuronal communication and regeneration of lost nerve tissues. This set of strategies is called neuroregeneration. In this area, the most studied biomaterials have been polymers compatible with the central nervous system. This is not an easy task, because any traumatic injury disturbs the physicochemical balance of the spinal cord. However, a compatible material implanted in these new conditions may slow the undesirable secondary damage process and, moreover, provide a means of communication between the chemical-ionic pulses on either side of the lesion. From this point of view, a chemically biocompatible polymer with the ability to transfer electric charges seems a good choice from among the materials studied.

Many materials have been tested in this context, some with the intention of providing structures where the cells grow to join the two ends of the severed spinal cord, while others have been used to stimulate axonal or cell growth in combination with the body itself [3-4], or to respond to the ionic impulses of neurons [5]. Some biomaterials applied to date in spinal cord injuries are:

- Carbon filament implants used as bridges for the axon growth
- Water-soluble polymers with ability to adhere to nervous tissue
- Biodegradable polymer microspheres containing nerve growth factors
- Biocompatible hydrogels
- Mixed reabsorbable polymers and oligomers made of lactic polyacid
- Semiconductor polymers derived from polypyrrole, polythiophene and lactic polyacid
- Semiconductor polypyrroles doped with halogens, synthesized by plasma. These studies have been made by the authors of this work [6]

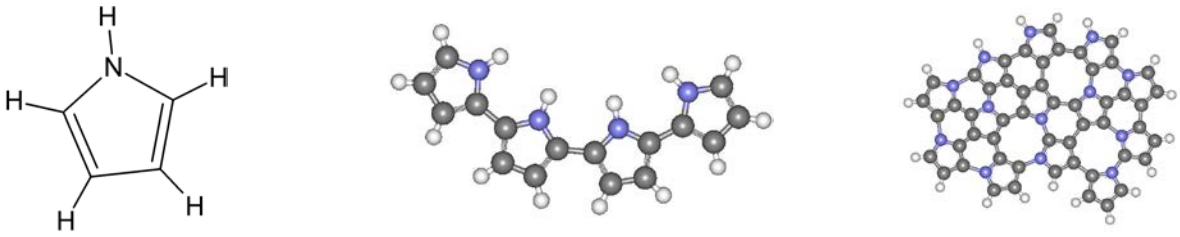
7. Semiconducting polymers

Most known polymers are electrical insulators. However, polymers can be prepared with structural features that facilitate the transport of electric charges. An important condition in this task is to alternate single and multiple chemical bonds along the atoms. The simplest example is the combination of carbon atoms linked by double and single bonds of polyacetylene (-CH=CH-CH=CH-) [7,8]. This kind of conjugated structure could transfer electrical charges. However, it does not mean that the electrons travel throughout the conjugated area, but an electronic disruption in the conjugation at one end could travel along the chain under the influence of electric fields. However, if the conjugation is broken somewhere by the consecutive repetition of simple or multiple bonds (C-C-C or C=C=C), charge transport also stops.

Another way to promote transport of electrical charges in polymers is to add materials with electronic density different to that of the main chain [9]. For example, halogens have a high electronic density because they have seven electrons in its valence layer and are available to add an outer electron to complete its octet. Atoms of this type that are spread across a chain of carbon produce polar sites that stimulate the transport of electrical charges. Both chlorine and iodine are halogens with these characteristics that could be added to polymers to facilitate electric charge movement [10,11]. Both techniques, conjugated bonds and polar elements, can be combined to produce biocompatible polymers with different abilities in the transport of electrical charges.

Polypyrrole (PPy) has been previously studied as a biomaterial in promoting growth, proliferation and cell differentiation in tissues such as liver and as support material for the manufacturing of artificial muscles [12,13]. On another hand, Iodine is an element that has been used medicinally for many years to disinfect wounds. Because of these attributes, polypyrrole and iodine were selected to synthesize semiconducting biocompatible polymers with the nervous tissue of the spinal cord with the purpose of implanting it to promote neuroprotection after a severe injury.

The structure of pyrrole is formed by a heteroaromatic 5-membered ring, four carbons and one nitrogen surrounded by hydrogens (-CH=CH-CH=CH-NH-). The NH group is generically known as amine and is in many cell fluids, particularly in the spinal cord. From a spatial standpoint, the pyrrole ring forms a plane with double and single bonds interrupted by the nitrogen atom of the amine. In forming the polymer, the rings lose hydrogen atoms reconnecting the points left by the absences (Figure 4). Depending on the point of attachment, this process can form large sequences of rings with conjugated double and single bonds, thus building semiconducting polymers.



Pyrrole Linear Polypyrrole Networked Polypyrrole

Figure 4. Structural Models of pyrrole and polypyrroles in linear and crosslinked configurations.

8. Synthesis of polymers by plasma

The synthesis method is crucial in the construction of semiconducting polymers compatible with the central nervous system. Because, although there is the formation of long sequences of conjugated

bonds during polymerization, if harmful compounds with the spinal cord tissues intervene in the process, the polymer may not be compatible with the chemistry of these tissues due to the amount of residuals left by accelerators, solvents or intermediary products, typical of chemical reactions. Therefore, the method of polymerization should not use another compound, except the monomer, in order to reduce undesirable or adverse reactions in the body with the implant.

A technique that meets these characteristics is plasma polymerization where the chemical reactions are carried out with electric discharges and are promoted by the constant collisions between the monomer, electrons and ions accelerated by electric fields. The kinetic energy that electrons reach during the polymerizations can be equivalent up to 100,000°C in the known thermodynamic temperature [14]. However, since the electronic mass is very small, the average thermodynamic temperature during the chemical reactions, considering also much heavier chemical agents, may not exceed 60°C. In these conditions, the energy of the accelerated electrons in the plasma can be similar to the bonding energy of the monomers in sufficient magnitude to excite or break chemical bonds promoting the formation of the polymer [15].

The initiators and accelerators of chemical reactions are not chemical but electric potentials and fluxes of electrons and ions that occur during electric discharges. The only chemicals involved in these processes are reactive molecules and polymers with different growth, all derived from or on base monomers.

As the electronic structure of pyrrole is very homogenous, collisions with plasma reactive particles are carried out on nearly all the atoms in the ring. This causes the chain growth to take place in any of their atoms, which can result in three-dimensional structures of crosslinked polymers and not necessarily in linear chains. With these features, once implanted in the spinal cord, the tissue and fluids will interact with crosslinked polymer particles swelling them in solid-liquid systems. However, a consequence of these conditions of synthesis is that the electrical properties of plasma polymers can be reduced compared to the same polymers synthesized by other methods. To overcome this disadvantage, these polymers were combined with other compounds to create polar sites to assist in the transport of electrical charges. This process is called doping.

As with plasma polymerization, plasma doping is promoted by ionization and collisions of particles in the plasma and, depending on the power applied to the system, can produce chemical bonds between the dopant and polymer chains. At the end of this process of simultaneous polymerization and doping by plasma, doped polymers have a higher electrical conductivity than their counterparts without doping.

Considering the above, several polymers and copolymers with pyrrole and iodine were synthesized for use as implants in the spinal cord of rats with complete transection lesions. The syntheses were carried out in gas-phase produced by plasma glow discharges in radio frequency resistive mode (Figure 5).

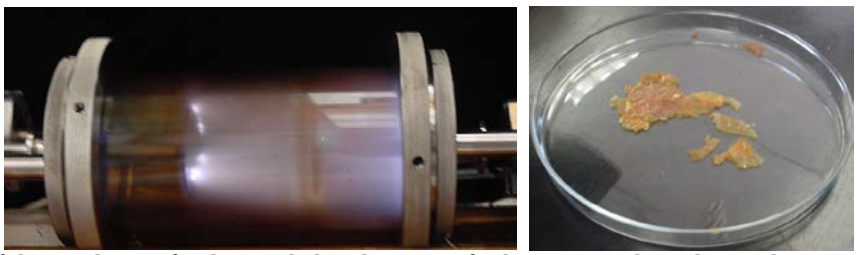
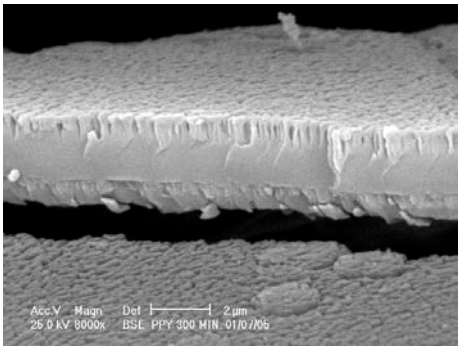


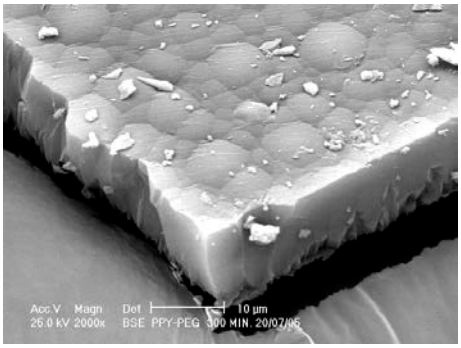
Figure 5. Images of the synthesis of polypyrrole by plasma. Left: the reactor where the synthesis took place; right: thin films obtained at the end of the reaction.

Pyrrole-polyethylene glycol copolymers (PPy-PEG) were also synthesized combining pyrrole amines and glycols oxygenated groups. Although the structure of ethylene glycol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$) has no double bonds to facilitate the transport of electric charges, the objective was to increase the hydrophilicity of polypyrroles through the insertion of OH hydrophilic groups [16]. The copolymers were formed with pyrrole and polyethylene glycol with molecular weight of 600 amu.

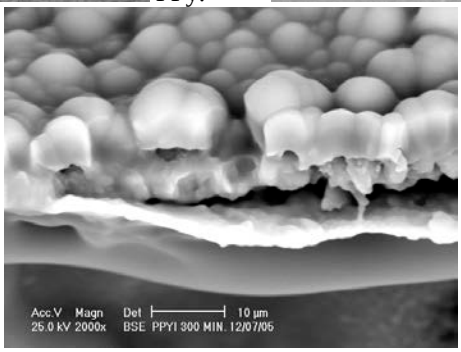
The polymers were formed in thin films with thicknesses between 1 and 20 μm with rough morphology, whose protrusions increased with the iodine doping. The morphology of polymers is important because the roughness may increase the surface contact of the material with the fluids and tissues of the spinal cord (Figure 6).



PPy.



PPy-PEG.



PPy-I.

Figure 6. Micrographs of electronic microscopy where the morphology of polypyrrole (PPy) Polypyrrole-Polyethylene glycol (PPy-PEG) and polypyrrole doped with iodine (PPy-I) film is shown. Note the difference in the roughness of surfaces.

The analysis of infrared and X-ray photoelectron spectroscopy suggests that there are multiple bonds (C=C and C≡N) in the polymers. These bonds may be caused by energetic particles from the plasma that fragments the CH groups of molecules generating radicals that recombine to form multiple bonds. However, these same energetic particles also produce fractures in the pyrrole molecules which result in aliphatic fragments incorporated into the polymer networks. This is noted in the atomic content of carbon and nitrogen in the plasma polypyrroles, which stoichiometrically should be C/N = 4, but whose actual relationship is about 8. From a global perspective, this means that half of the material could be made of pyrrole rings and the other half of their derivatives and oxygenated compounds.

As part of the material characterization, the electrical conductivity in PPy was calculated according to the relative humidity, which resulted in the range of 10^{-12} to 10^{-10} S/cm, gradually increasing two orders of magnitude in contact with the environmental humidity (Figure 7). These values lie at PPy

in the area of insulating materials. However, when PPy is combined with iodine, PPy-I, its conductivity increased to the 10^{-10}-10^{-4} S/cm range in two regions with different pattern. At low relative humidity, 10% to 75%, conductivity varies little, around 10^{-10} S/cm, similar to the highest values of PPy. However, after 75% relative humidity, conductivity increases rapidly up to six orders of magnitude reaching up to 10^{-4} S/cm [17-19].
Because the polymerization takes place in the gas phase, it can be seen that the iodine is evenly distributed in PPy-I. On average, there is one iodine atom for every 7 or 8 pyrrole rings. This number indicates that both polymers are essentially polypyrroles, but that a small amount of iodine is sufficient to increase the conductivity at the levels described only when there is a large amount of water in the neighborhood. This behavior suggests that PPy-I in contact with the fluids of the spinal cord (which contains water and salts) its conductivity increases by several orders of magnitude, facilitating the transport of charges in the material after being implanted.

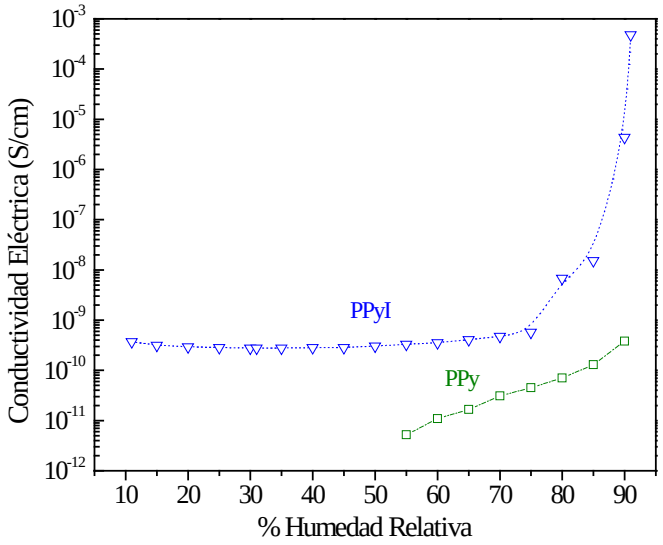


Figure 7. Electric Conductivity of PPy and PPy-I as function of relative humidity.

9. Semiconducting polymer implants in injured spinal cords

In the biological study, Long Evans rats of 250-300 g and 2 months of age were used. The rats were anesthetized and a complete cut of the spinal cord was made (Figure 8). For this, a longitudinal section in the thoracic region through the skin was performed, the paraspinal muscles were dissected, the spinous apofisis of thoracic vertebrae 9 was removed and its bone plate that covers the spinal cord was broken at that level. Meninges were opened longitudinally and a cross-section cut was performed in the spinal cord severing all nerve tissues at that level. This maneuver resulted in injury that paralyzed the hind legs of the rats.
Several groups of rats formed in sets of 3-6 animals were created to implant PPy, PPy-I and PPy-PEG in the injury site in order to study the response of the spinal cord to each material. Another group of rats was left and nothing was implanted in order to serve as control.
After the surgery, all rats were administered analgesics and antibiotics and were placed in an intensive care unit for small animals with controlled temperature and humidity. Once recovered from anesthesia, animals were placed in individual boxes and taken to a specialized animal laboratory where they were kept for 2 months.

Figure 8. Images that show the initial stage of surgery to produce a lesion in the spinal cord and the implant of the polymers.

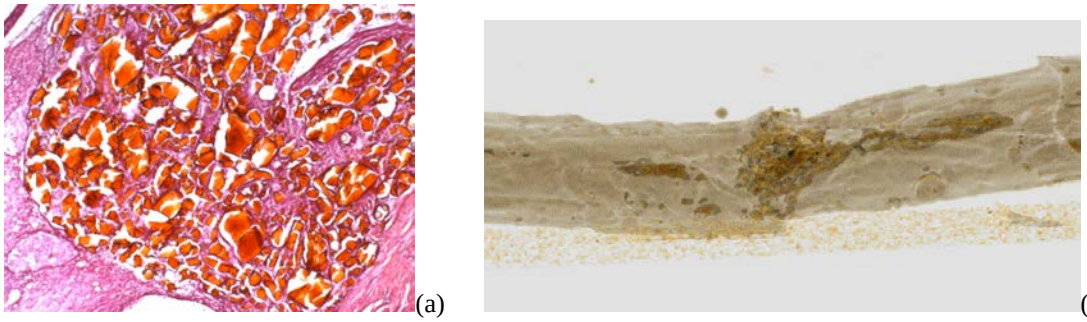
10. Organism response to implant

When a material is implanted in the body, the first biological response is the formation of a proteic layer around the foreign material in the first minutes after the implant. In the following hours, the cells of the body's defense system, neutrophils and macrophages, proliferate and migrate to act if the foreign material poses potential harm. In the following days, the fibroblasts synthesize collagen, surrounding the foreign material isolating it from the other cells. When this happens, depending on the particular response of each individual, several days have elapsed since the injury.

It's a race against time in which cells seek to isolate the foreign material as quickly as possible. So, the period between implantation and isolation is the maximum life span the material has to perform its function. Then, depending on their size and nature, its effect is neutralized and reduced to the zone enclosed by the surrounding collagen.

In the case of polymers implanted in the spinal cord, this period is crucial as it is when a cascade of processes happens simultaneously because of the spinal cord injury and the body's response to the implant. The animals that were injured but did not receive a polymeric implant presented the formation of cysts and scars that isolate the two segments of the injured spinal cord.

It is expected that these processes are modified in animals that were injured and implanted, partially decreasing the inflammatory response, or changing the chemical reactions at the site of lesion. These changes can give the cut ends of the spinal cord an opportunity to reconnect, at least in some points. It is therefore very important that the implant does not cause additional swelling (Figure 9) and somehow pass part of the electrical pulses between the two sections of the spine.



(a) (b)

Figure 9. (a) Image which shows a histological cut on a spinal cord implanted with PPy after 8 weeks of evolution. The polymers show very little inflammation in the implant site. 20x Amplification. (Histological Technique) (b) MRI that shows the implanted spinal cord from a longitudinal perspective, where the migration of the polymer can be observed.

11. Motor recovery evaluation in the hindlimbs of rats implanted with polymers

The evolution of lesions and the influence of polymers in them are very complex and not enough is known about them to discuss at length in this work. However, what can be studied is the outcome of the motor and functional recovery in rats. There is a qualitative scale to measure the voluntary movement of the joints in hind limbs of rodents. The scale assesses the movement of ankle, knee and hip and is called BBB by the initials of the surnames of its creators, Basso, Beattie and Bresnahan [20].

The scale consists of 22 degrees, where "0" represents absence of movement and the grade is progressively increasing with the movement of the joints up to "21", a normal pace level. In a hypothetical progressive recovery from total paralysis of the hind limbs, BBB=0, a test animal would travel the scale to reach an equilibrium level. The time it takes and the level of movement reached depends on its ability to recover. It should be noted that actual recoveries of the test rats do not cover the whole scale, but they move by periods at different speeds until they stop at some point in the scale.

BBB average response in the first 56 days after the lesion of the rats studied in this work is shown in Figure 10 [21]. There are four groups: Control, which are the injured rats without implants; PPy are injured rats implanted with polypyrrole, PPy-PEG, are injured rats implanted with the copolymer formed from pyrrole and polyethylene glycol, and finally, PPy-I, who are injured rats implanted with polypyrrole doped with iodine.

According to the BBB and the time scale, the movement recovery of the hind legs shows a linear trend. The variable "r" shown on the graph is the slope of the average. From a physical point of view, it represents the speed of recovery of the animal in light of changing daily BBB, r=BBB/day.

The results indicate that the evolution of all rats, with or without implant, is very similar during the first 3 weeks after injury, as evidenced by BBB values in the range of 0 to 1.5. It is possible that the physiological consequences of surgery and the injury itself dominate during this period and therefore the recovery is very similar. However, after week 4, the body's response to polymer implants begins to manifest.

The Control group is less likely to achieve recovery, r=0.051 with a maximum BBB=3, while the greatest recovery is in PPy-I, with r=0.1 and highest BBB=4.7. This means that the control group would take approximately 411 days to recover fully, if it maintained the same trend. The recovery of this group provides a natural evolution without implant of this type of injury and may serve as a baseline. The PPy-I group would need half the recovery time of the control group, since its speed is about double. For its part, PPy and PPy-PEG groups have a similar recovery rate, r=0.078 and r=0.069, respectively, with values approximately half of the control groups and PPy-I, with highest BBB of 3.6 and 4, respectively. The difference in the recovery of the study groups may be due to the structure of polymeric implants.

The deviation of the average points on the line exemplifies some acceleration and recession periods in the recovery of rats. As far as can be observed in the first 8 weeks after the lesions, there is not a clear reduction in the tendency to indicate that recovery is stabilizing at some point on the BBB scale.

The biological response in PPy and PPy-PEG groups is very similar promoting approximately the same additional recovery above the baseline controls. A chemical difference between these two polymers is that the PPy-PEG has a higher proportion of oxygen.

The structure of PPy-I indicates that it is more reactive in the spinal system, as the recovery of movement is much greater than that obtained with the other polymers. An important variable of

PPy-I is that iodine doping increases the electrical conductivity and can be an important material in the ionic interaction with the spinal cord pulses that increases its recovery.

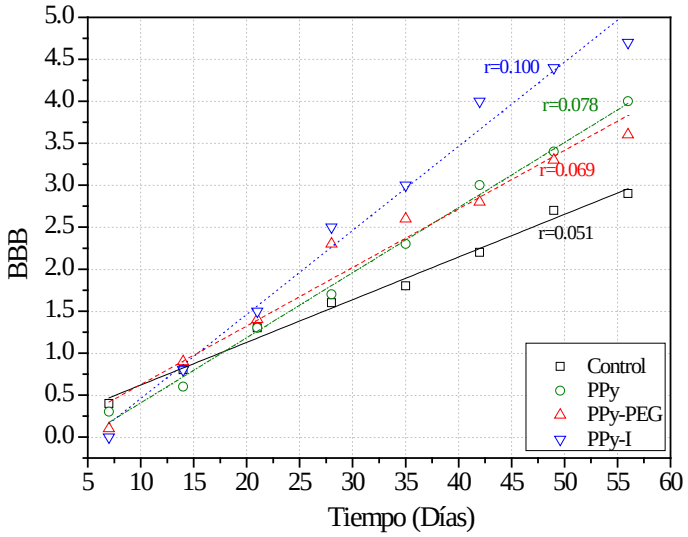


Figure 10. Average BBB of tested animal groups with complete section in the spinal cord (Control), or with implants of PPy, PPy-PEG or PPy-I. The motor recovery was evaluated during 56 days after the lesion.

12. Hindlimb movement of rats implanted with polymers

The animals were sacrificed after different periods of evolution for histological studies. The results show that most of the spinal cords of animals present a lot of scar tissue and cysts that complicate axonal reconnection and thus neuronal communication.

Injured rats in the control group have hindlimbs virtually paralyzed, involuntary movements occur only sporadically, they are unable to plant their feet on the floor, and only crawl. On average, rats in this group achieved a maximum rating of BBB=2.7 at week 8, which means they had limited movement in joints (Figure 11). However, this group has contrasting results as some rats of this group showed histological evidence of reconnection between the two ends of the severed spinal cord which was reflected in small movements in the hindlimbs of these animals.

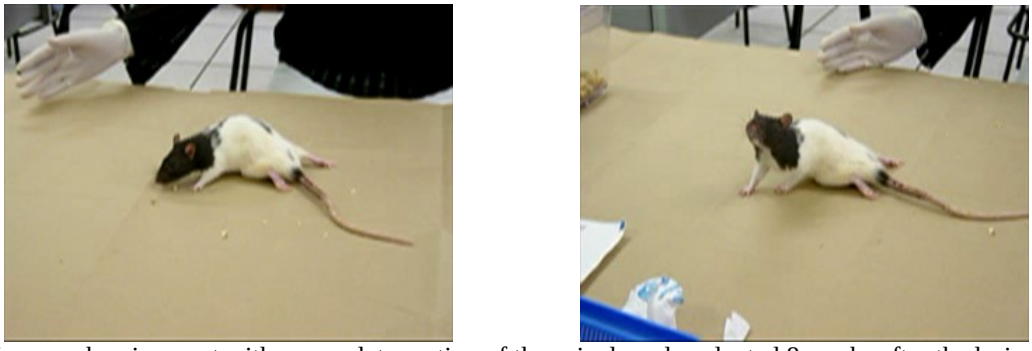


Figure 11. Images showing a rat with a complete section of the spinal cord evaluated 8 weeks after the lesion. Note how the position of the forelimbs is normal while the hindlimbs show lack of support.

On the other hand, animals that received implants with PPy and PPy-PEG had fewer and smaller cysts compared with control animals. They presented small pieces of reconnected tissue, allowing a small fraction of neuronal communication. This is reflected in the motor activity of the affected limbs, which developed a little better than in the control group because the animals could plant and move one foot, though with difficulty. Their average score in the eighth week after the injury was BBB=3.7, which means that they partially moved the joints of one leg (Figure 12).

Figure12. Images which show a rat with a complete section in the spinal cord with a PPy-PEG implant evaluated 8 weeks after the injury. The rats can plant one sole with voluntary although limited movement.

The group of animals that received PPy-I implants had a higher proportion of connective tissue than in the previous case and therefore the affected functions had a better recovery. Many animals in this group were able to place the soles of the two hindlimbs on the floor and some were able to climb obstacles using the hindlimbs, although with help from the forelimbs, so they generally recovered lost functions better than controls. Their BBB rating average after 8 weeks was 4.7, which means that they could move three joints of both hindlimbs (Figure 13).

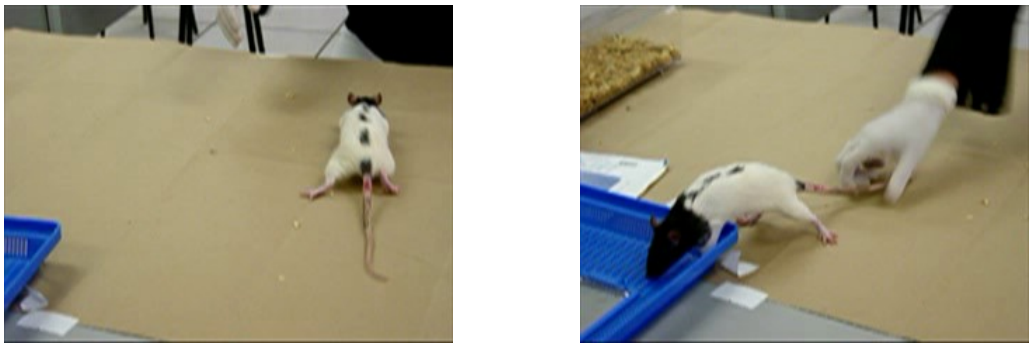


Figure 13. Images showing a rat with a total transection of the spinal cord, implanted with PPy/I, and evaluated 8 weeks after the injury. The animal can plant both soles and move voluntarily both hindlimbs to walk and climb obstacles.

It is important to note that, although the reconnection of tissue at the injury site is mentioned, its proportion to the rest of the spinal cord is very slight and can be linked to a neuroprotective effect of the implant. This effect was manifested to a greater extent in the groups of rats that received implants of PPy and PPy-I and was characterized by decreased inflammatory response and in a smaller amount of immune system cells at the site of injury, minor amount of scar tissue and fewer cysts [22-24].

13. Conclusions

Injuries to the spinal cord cause partial or total disability in people and so far a successful treatment to reverse its effects has not been developed yet, presumably due to its complexity and this problem must be approached from different scientific perspectives. This work reported the study of injuries produced by complete section of the spinal cord in rats from a multidisciplinary perspective that includes medicine, biology, chemistry, physics, materials science and bionics. The complete section of the spinal cord is probably the most severe case of spinal cord injury known.

Because neuronal communication takes place by chemical ion pulses, biocompatible polymers were designed with the ability to transfer electric charges interacting with moisture and in its synthesis not involve chemicals which are potentially harmful to the spinal cord. The materials formed networks of polymers with pyrrole, polyethylene glycol and iodine under resistive RF glow discharges. The only chemicals involved in these processes are monomers and their derivatives formed during the polymerization. The resulting polymers are biocompatible and have low electrical conductivity, to the extent that they can be considered insulators, but in contact with humidity increase their conductivity by several orders of magnitude.

The polymers were implanted in rats of the Long Evans strain which underwent a surgical lesion of complete transection of the spinal cord at thoracic level 9, which paralyzed both hind limbs. Immediately after the injury, the rats were implanted with test polymers and provided postoperative care. The motor recovery of rats for 8 weeks after injury was studied.

The results show that the injured rats that received no implant had paralyzed hindlimbs, with movement only in the forelimbs. Rats implanted with pyrrole polymers and copolymers showed limited movement in one hindlimb, their progress was driven by three limbs with slow movement. Finally, rats receiving implants of PPy-I had broad movement in both hindlimbs, their movement being much faster and coordinated than in the above cases.

These results indicate that the application of implants of polypyrrole synthesized by plasma in rats with complete transection of the spinal cord promotes motor functional recovery in animals. The mean level of recovery achieved a breakthrough in the field of spinal cord injury. There was no similar recovery so far reported in lesions of this severity, which usually produce total and irreversible paralysis.

14. Prospects

There is still a long way to go in regards to the application of these results in human patients with spinal cord injuries. The next step of this work is to implement the biopolymers in primates. If enough successful results are achieved, the next stage would be to study the human response. However, this is only part of the work, since another task ahead will be to address the case of spinal cord injury by contusion, which represent the type of traumatic spinal cord injury more common in humans and requires different therapeutic strategies.

The application of polymers synthesized by plasma with potential to transfer electrical charges in spinal cord injuries is in its initial stage. At this point, a new generation of biomaterials and implant techniques will probably be developed that will improve the recovery of functions lost as a result of such injuries. A final observation is to highlight the need for collaboration between different scientific disciplines to find solutions to complex health problems that the traumatic spinal cord injury represents.

Acknowledgements

To the Mexican institutions participating in this work: Instituto Nacional de Investigaciones Nucleares; Universidad Autónoma Metropolitana Iztapalapa; Instituto Nacional de Neurología y Neurocirugía; Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, and Proyecto Camina A.C. All of them have intensively participated in the tasks of this work. A special acknowledgement also to Conacyt for the partial financial support through the projects 80735 and 47467.

References

1 - Diaz-Ruiz A., Zavala C., Montes S., Ortiz-Plata A., Salgado-Ceballos H., Orozco-Suarez S., Nava-Ruiz C., Pérez-Neri I., Perez-Severiano F., Ríos C. Antioxidant, antiinflammatory and antiapoptotic effects of dapsone in a model of brain ischemia / reperfusion in rats. *Journal of Neurosciences Research*, **86(15)**, 3410-3419, 2008.
2 - Beattie M.S., Hermann G.E., Rogers R.C., Bresnahan J.C. Cell death in models of spinal cord injury. *Progress in Brain Research*, **137**, 37-47, 2002.
3 - Xiang S., Pan W., Kastin A.J. Strategies to create a regenerating environment for the injured spinal cord. *Current Pharmaceutical Design*, **11**, 1267-1277, 2005.
4 - Barakat D.J., Gaglani S.M., Neravetla S.R., Sanchez A.R., Andrade C.M., Pressman Y., Puzis R., Garg M.S., Bunge M.B. and Pearse D.D. Survival, Integration, and Axon Growth Support of Glia Transplanted Into the Chronically Contused Spinal Cord. *Cell Transplant*, **14**, 225-240, 2005.
5 - Olayo R., Ríos C., Salgado-Ceballos H., Cruz G.J., Morales J., Olayo M.G., Alcaraz-Zubeldia M., Alvarez A.L., Mondragon R., Morales A., Diaz-Ruiz A. Tissue spinal cord response in rats after implants of polypyrrole and polyethylene glycol obtained by plasma. *Journal of Materials Science: Materials in Medicine*, **19(2),** 817-826, 2008.
6 - Olayo R., Morales J., Mondragón R., Álvarez A.L., Morales C.A., Ríos L.C., Díaz M.A., Cruz G.J., Olayo M.G., Salgado H. Uso de polímeros derivados de pirrol sintetizados por plasma para la neuroprotección y reconexión del sistema nervioso central. Patente IMPI, PA/2005/013415, México, 2005.
7 - Vásquez O.M. Síntesis y caracterización de poliacetileno por plasma. Tesis de Maestría en Ciencias con Especialidad en Ingeniería Química. Escuela Superior de Ingeniería Química, Instituto Politécnico Nacional, D.F., 2005.
8 - Cruz G.J., Olayo M.G., Fernández G., Vásquez M., Morales J., Olayo R. Micro and meso structures in plasma polymers of trichloro ethylene. *IEEE Transactions on Plasma Science*, **37-9**, 1675-1682, 2009.
9 - Palacios G.J.C. Estudio sobre las Propiedades Eléctricas de Películas Delgadas de Polianilina y Polipirrol Polimerizadas por Plasma. Tesis de doctorado en Ciencia de Materiales. Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Mex., 2005.
10 - Olayo M.G., Morales J., Cruz G.J., Olayo R., Ordoñez E., Barocio S.R. On the influence of electron energy on iodine doped polyaniline formation by plasma polymerization. *Journal of Polymer Science, Part B: Polymer Physics*, **39-1**, 175-183, 2001.
11 - Olayo M.G., Cruz G.J., Ordóñez E., Morales J., Olayo R. Molecular simulation of plasma polymerized polyaniline-iodine compounds. *Polymer*, **45**, 3565-3575, 2004.

12 - Morales J., Osorio C., Montiel R., Vázquez H., Olayo R., Olayo M.G., Cruz G.J., Perez E. Autoensamble de capas de polímeros iónicos sobre polietileno funcionalizado por plasma de pirrol. *Superficies y Vacío*, **21(3)**, 1-4, 2008.
13 - Morales J., Pérez-Tejada E., Montiel R., Vázquez H., Olayo R., Gómez L.E., Gutiérrez M.C., Olayo M.G., Cruz G.J. La Física Biológica en México: Temas Selectos 2, Capítulo 10, Modificación Superficial por Plasma Aplicada a Biomateriales. Ed. El Colegio Nacional, 1ª Edición, 241-258, 2008.
14 - Olayo M.G., Morales J., Cruz G.J., Barocio S.R., Olayo R. Numerical and experimental analysis of the plasma in the synthesis of polyaniline. *Journal of Polymer Science, Part B: Polymer Physics*, **41**, 1501-1508, 2003.
15 - Cruz G.J., Morales J., Castillo-Ortega M.M., Olayo R. Synthesis of polyaniline films by plasma polymerization. *Synthetic Metals*, **88,** 213-218, 1997.
16 - Gomez L.M., Morales M.P., Cruz G.J., Olayo M.G., Palacios J.C., Morales J., Olayo R. Plasma Copolymerization of Ethylenglycol and Allylamine. *Macromolecular Symposia*, **283-284**, 7-12, 2009.
17 - Cruz G.J., Morales J., Olayo R. Films obtained by plasma polymerization of pyrrole. *Thin Solid Films*, **342/1-2**, 119-126, 1999.
18 - Morales J., Olayo M.G., Cruz G.J., Castillo-Ortega M.M., Olayo R. Electronic conductivity of pyrrole and aniline thin films polymerized by plasma. *Journal of Polymer Science, Part B: Polymer Physics*, **38**, 3247-3255, 2000.
19 - Colín E., Olayo M.G., Cruz G.J., Carapia L., Morales J., Olayo R. Affinity of amined plasma polymers with ionic solutions similar to the human body. *Progress in Organic Coatings*, **64**, 322, 2009.
20 - Basso D.M., Beattie M.S., Bresnahan J.C. A sensitive and reliable locomotor rating scale for openfield testing in rats. *Journal of Neurotrauma*, **12**, 1-21, 1995.
21 - Álvarez A.L. Influencia del campo magnético sobre la regeneración axonal después de un implante de polímero semiconductor en un modelo de sección completa de médula espinal. Tesis de Maestría en Ingeniería Biomédica, Universidad Autónoma Metropolitana Iztapalapa, D.F., México, 2008.
22 - Olayo R., Álvarez L., Lozano R., Escalona A., Morales C., Morales J., Olayo M.G., Cruz G.J., Ríos C., Díaz-Ruiz A., Salgado Ceballos H. La Física Biológica en México: Temas Selectos 2, Capítulo 8, Implante de Polímeros Sintetizados por Plasma en Lesiones de Médula, Ed. El Colegio Nacional, 1ª Edición, 198-206, México, 2008.
23 - Mondragón L.R. Evaluación del efecto de tres implantes poliméricos en la función nerviosa en un modelo de lesión por sección completa de la médula espinal en ratas. Tesis de Maestría en Ingeniería Biomédica, Universidad Autónoma Metropolitana Iztapalapa, D.F., México, 2009.
24 - Álvarez A.L., Salgado-Ceballos H., Morales J., Ríos C., Díaz A., Cruz G.J., Olayo M.G., Mondragón R., Morales A., Escalona A., Godínez R., Verdugo L., Olayo R. Influencia del campo magnético e implantes de polímero semiconductor sobre la regeneración axonal en un modelo de lesión traumática de médula espinal. IV Latin American Congress on Biomedical Engineering 2007, Bioengineering Solutions for Latin America Health, *IFMBE Proceedings* **18**, 646–649, Springer-Verlag, Berlin, Heidelberg 2007.