

# Materials directed to implants for repairing Central Nervous System

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Central Nervous System (CNS) can be damaged by a wide range of injuries and disorders which entail permanent disability in some cases. Moreover, CNS repairing process presents some complications. The natural repair mechanism, which consists on the glial scar formation, is triggered by the inflammatory process. Molecules delivered during these processes, inflammation and glial scar formation as well as oxygen and glucose deficiencies due to the injury, create an inhibitory environment for axon regeneration and remyelination which is known as "secondary injury".

Biomaterials are taking up an even more important role in repairing CNS. Physicochemical properties of some ceramic materials have inspired different applications to repair CNS as substrates, electrodes or molecule vehicles. Based on their biocompatibility, capability to neutralize reactive species involved in the inflammatory processes and their versatile processing to obtain scaffolds with different shapes and sizes, ceramics are a succulent offer in nervous tissue engineering. Furthermore, their possibilities have been increased with polymeric-ceramics composites development, which have given rise to new interesting horizon.

*Keywords: Biomaterials, Bioceramics, Oxides, anti-inflammatory, electrodes for electro-stimulation, CNS.*

## Materiales dirigidos a implantes para reparar el Sistema Nervioso Central

Existen diferentes tipos de lesiones o desordenes del Sistema Nervioso Central (SNC) que pueden provocar graves secuelas e incluso en algunos casos una discapacidad permanente. Además, el proceso de reparación del SNC tiene algunas complicaciones. El mecanismo natural de reacción a una lesión, el cual consiste en la formación de una cicatriz glial, es desencadenado por un proceso inflamatorio. Las moléculas liberadas durante estos procesos, la inflamación y formación de la cicatriz glial, así como la deficiencia en oxígeno y glucosa debidos a la lesión, crean un ambiente que inhibe la regeneración axonal creando la llamada "lesión secundaria".

Los biomateriales están adquiriendo un papel cada vez más importante en la reparación de SNC. Las propiedades físico-químicas de algunos materiales han inspirado el uso de los mismos en diferentes aplicaciones para la reparación del SNC como sustratos, electrodos o moléculas transportadoras. En base a su biocompatibilidad, capacidad de neutralizar especies reactivas implicadas en el proceso inflamatorio y a la gran cantidad de posibilidades del procesamiento para obtener andamiajes con diferentes formas y tamaños, los materiales cerámicos suponen una suculenta oferta en la ingeniería del tejido nervioso. Además sus posibilidades han aumentado con el desarrollo de composites polimérico-cerámicos, los cuales ha abierto un nuevo e interesante horizonte en la materia.

*Palabras clave: Biomateriales, Biocerámicas, óxidos, anti-inflamación, electrodos para electro-estimulación, SNC.*

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## 1. INTRODUCTION

Injuries in the Central Nervous System (CNS) can be caused by infections, hypoxia, poisoning, stroke, degenerative illness or traumas (1). Traumas on spinal cord create irreversible injuries which entail permanent incapacity. Approximately 2.000.000 people worldwide, the most of them with ages lower than 30 years old, suffer some kind of injury in the spinal cord. Every year 13.000 new cases are added (2). This trauma can be caused by a contusion, compression or extension. Injury and causal processes limit both oxygen and glucose contribution to the tissue promoting neuronal ischemic cell death, axon damage and demyelination. This is the so-called "secondary injury", the main cause of the post-traumatic

neural degeneration itself. Subsequently, the traumatic process is followed by the inflammatory process associated with glial activation, release of inflammatory factors and cytokines, and scar formation that impedes axons to re-grow and that aggravates the progression of the damage (3).

Low impact injuries in Peripheral Nervous System (PNS) heal by formation of fibrin cable across the gap (4). This allows Schwann cells eventually migrate from both nerve ends, thereby orienting Bungner bands to promote neurite outgrowth (5). It has been found nerve cells are capable of easily bridging gaps narrower than 6 mm (6). Natural repair inspire researchers to investigate a suitable strategy for enhancing the

formation of Bungner bands. In the framework of this research it was identified that microstructure like filaments provide a better topography to promote Bungner band formation (4,5,7). However, in the case of CNS, spontaneous regeneration is impossible due to the inhibitory environment created by glial scar formation and accumulation of myelin-associated inhibitors (8,9). In this environment, axon regeneration fails, and remyelination may also be unsuccessful. The glial reaction to injury recruits microglia, oligodendrocyte precursors, meningeal cells, astrocytes and stem cells. Damaged CNS also contains oligodendrocytes and myelin debris. Most of these cell types produce molecules that have shown inhibitory effect for axon regeneration (10). Moreover, the primary injury in CNS expands further by damaging the nerve cells due to secretion of free radicals from the blood via the blood-brain barrier resulting in secondary injury previously mentioned (11). It means that glial scar formation, which is a natural therapeutic process developed by our own body to repair CNS, become a loophole for the cells around the injury.

## 2. MATERIALS

The main roles pursued by biomaterials in CNS injury treatments are three; first, neuroprotection by means of decreasing inflammation, exocitotoxicity, apoptosis y secondary cell death processes. Second, is cell transplantation or tissue engineering to replace damaged cell or tissues and provides sustained delivery protein. Finally, it is cell regeneration and reorganization promoting axonal and neurite growth. With this aim, biomaterials are getting an even more important role for the treatment of disorders and injuries of the CNS and PNS offering different strategies to deal with CNS injury treatments (1):

1. Materials used as substrates for cellular support, axonal regeneration and dendrite growth.
2. Materials to control the inflammatory response associated to an injury process, avoiding the secondary cell death, and the implant rejection.
3. Materials used as electrodes to electrostimulate directional axon growth of neurons.
4. Materials used as delivery systems with different objectives.

### 2.1. Substrates: Conduits and Scaffolds.

The implants suggested as substrate for neural tissue regeneration can be enclosed in two groups depending on the geometry: connection materials with tubular shape (conduits) and three dimensional networks (scaffolds).

Connection materials, commonly named conduits, have tubular shape. Their aim has been focused to connect opposite sides of damaged peripheral nerve working as bridge tissue regeneration and finally the neural reconnection. The properties of a suitable conduit are the following it requires a slim size, suitable porosity (which allow cell colonization, vascularization and nutrients arrival), biocompatibility, they should be neuroconductive, neuroinductive, infection resistant and they should feature suitable physicochemical properties on the surface (avoiding degradation under electrostimulation conditions) and the right mechanical properties (similar to

the tissue to avoid damages in the healthy or new generated tissue). Finally, permeability is an indispensable property which allows the arrival of nutrients to the tissue during the therapeutically process (7,12).

In other hand, scaffolds are oriented to fill the cavity generated in the CNS during a damaging process are also necessary. The objectives of the scaffold when it is implanted in an injury zone are acting as cell support round the injury and as substrate for cellular growth, axonal regeneration and neurites growth. The requirements of an ideal scaffold are the following, biocompatibility, low inflammatory response, controlled porosity which allows vascularization and cell migration and a pore distribution and surface texture that mimic the extracellular matrix (ECM), they should be neuroconductive, neuroinductive and possess suitable mechanical (flexibility and hardness) and morphological properties (7,13). There are two types of scaffolds reported, gels and sponges, which are the most commonly used. Gels are polymers designed to mimic the ECM controlling the tissue structure, nutrients distribution, metabolites and soluble factors. On other way, sponges are porous tridimensional structures (1). Scaffolds can be implanted seeded with stem cells, susceptible of a future differentiation that generally depends on the physiological conditions in which are involved (14). Scaffolds can be modified mimicking ECM to improve cells differentiation and growth (15), favoring their integration, survival, adhesion, connection and extension. Integrin, peptides and proteins can be added to the scaffold to aid cell adhesion.

One type of materials commonly used as scaffolds, focused on the CNS and PNS, are polymers. There are a wide number of polymers that match the conditions required: non-toxicity, anti-inflammatory and mechanical properties and porosity. Among them there are natural polymers (chitosan, chitin, collagen, gelatin and alginate), biodegradable synthetic polymers poly (lactic-co-glycolic acid) (PLGA), poly  $\epsilon$ -caprolactone (PCL), polylactic acid (PLLA) and conductive polymers (polypirrol y polyaniline) (7). There are numerous techniques to shape polymers to obtain scaffolds: self-assembly nanofibres, solvent casting and particulate leaching, gas foaming, emulsification/ freeze-drying, liquid-liquid phase separation, electrospinning and computer-aided design (7). The technique used to shape scaffolds should allow simulating the physical properties of an ECM, for example, obtaining tubular structures which mimic microtubules, axons and dendrites (1). One example are the self-assembly scaffolds with L-amino acids. Morphology plays an important role in the cellular development too. Depending on the distance between fibers, neurites can growth across or in the fibers direction (16). Moreover, topographic accidents as grooves, hills chain, pores or nodules, can act as guides which affect adhesion, migration, proliferation and cellular differentiation of new tissue (17). The wettability is other important parameter, related with the cellular adhesion. If material surface is hydrophilic, then cells will have more probability to make contact and attach to the surface (18,19).

In the last years, hydrogels are the main materials developed as scaffolds to be used to repair nervous tissue (7). It is due to the chemical, mechanical and dimensional properties which are similar to ECM (20). For this reason, they are an exceptional support for neurites and axon growth (21), cellular proliferation and differentiation (7). Other advantage of hydrogels is the possibility to be directly injected in the lesion site avoiding the complications of a surgery. One hydrogel commonly

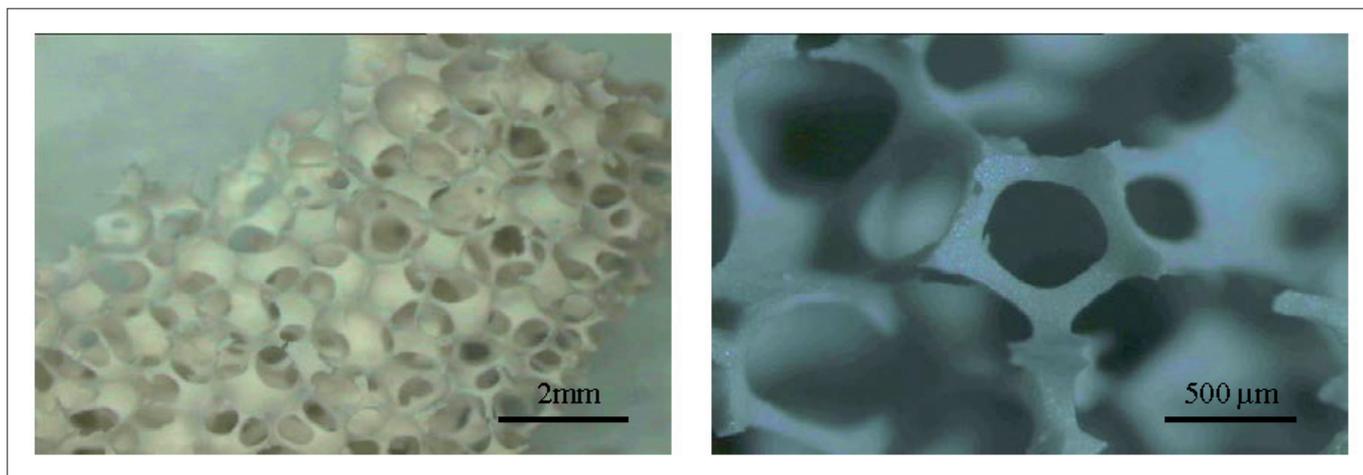


Figure 1. Ceramic TiO<sub>2</sub> processed as scaffold with macroporosity (26).

used in CNS tissue repairing is poly(*N*-(2-hydroxypropyl) methacrylamide) (PHPMA) (22), which is biocompatible and possesses neuroinductive and neuroconductive properties. Other synthetic hydrogels commonly used as scaffolds are: poly(ethylene oxide) (PEO), polyvinyl alcohol (PVA), polyacrylic acid (PAA), polypropylene (fumarate-co-ethylene glycol) (P(PF-co-EG)) and polypeptides (23).

Although, polymers and hydrogels have been heading the list of materials used in nervous tissue regeneration, the great biocompatibility showed by ceramic materials and the big impulse of polymeric-ceramic-composites during the last years in biomaterials field have opened a new research line dedicated to nervous tissue regeneration (24). Major advantages of ceramic materials are their good biocompatibility, high hardness and improved corrosion and wear-resistance properties. However, similar mechanical properties between tissue, soft tissue in case of CNS, and material implanted are convenient. These mechanical problems can be solved with polymer addition making a hybrid material. Ceramic processing field is advancing nowadays allowing the developing of ceramic materials with multiple shapes, sizes and porosity **Fig. 1**. There are several ceramic processes to obtain scaffolds as, for instance, foam replication method, sacrificial template method and direct foam method, between the most common. While there are multiple techniques to process bioceramics, polymer addition has multiplied these possibilities. The ceramic material can be dispersed on a polymer matrix processed by different methods. In the opposite side, the ceramic scaffold can be coated or impregnated by different methods with a polymer (25).

On the other hand, carbon nanotubes and nanofibers play an important role in the search of new biomaterials due to their mechanical, optical, electrical and structural properties. In tissue regeneration these materials start to wake up an increased interest regarding to their modification with the aim to be integrated in the human body (27).

## 2.2. Avoiding inflammatory response

As mentioned, one of the main problems of CNS injuries is the inflammatory response. In mammalian cells there are nitrogen and oxygen-based pro-oxidant molecules, while in some eukaryotic cells these molecules are in the form of radicals: superoxide radical (O<sub>2</sub><sup>•-</sup>), nitroxide radical (NO<sup>•</sup>),

hydroxyl radical (OH<sup>•</sup>) and peroxynitrite (OONO<sup>•</sup>) (28). This kind of molecules possesses an unpaired electron responsible of their high chemical reactivity as these molecules are susceptible to donate this electron or to accept a new one to reach electronic stabilization. Other kinds of molecules as H<sub>2</sub>O<sub>2</sub>, which are not radical species, are also characterized because of its high reactivity. Cells have enzymatic systems which keep redox balance in normal levels avoiding the so-called "oxidative stress". Nevertheless, sometimes there are disorders of this redox balance that trigger the inflammatory process and that are the cause of several pathologies (29).

When our organism suffers an injury, the first response is the recruitment of inflammatory cells. Among them, the first cells arriving at the injury site are the polymorph nuclear neutrophils which secrete reactive molecules of oxygen and nitrogen (ROS, RNS). High concentrations of intracellular Ca<sup>2+</sup> activates these cells, which is translated in an increase of some integrin expression and the respiratory combustion. The inflammatory process is successfully solved when the cellular death is produced by apoptosis, and then, takes place the phagocytosis. In the worst case, cellular death is resolved through necrosis which promotes an increase of pro-inflammatory mediators and destabilizes the redox balance. The inflammatory process is in a feedback (29,30).

In other hand, the inflammatory process can be associated to the implant. When the organism detects a foreign body, for example, the implant, the final aim of the inflammatory process is the encapsulation of the foreign body to isolate it from healthy tissues avoiding their infection. The duration and magnitude of inflammation process, determines the stabilization and biocompatibility of this material, ending in the worst case with its encapsulation and rejection.

For this reason, is necessary that any implant introduced to repair an injury does not promote an inflammatory mechanism, which can be translated in its rejection. Furthermore, it would be desirable the material could cushion the inflammatory process already activated by an injury.

### 2.2.1 TITANIUM OXIDES

In last decades, metallic titanium has been successfully imposed in the biomaterials industry used in dental implants and orthopedic prosthesis (31). This material shows excellent biocompatibility due to the thin surface layer of titanium

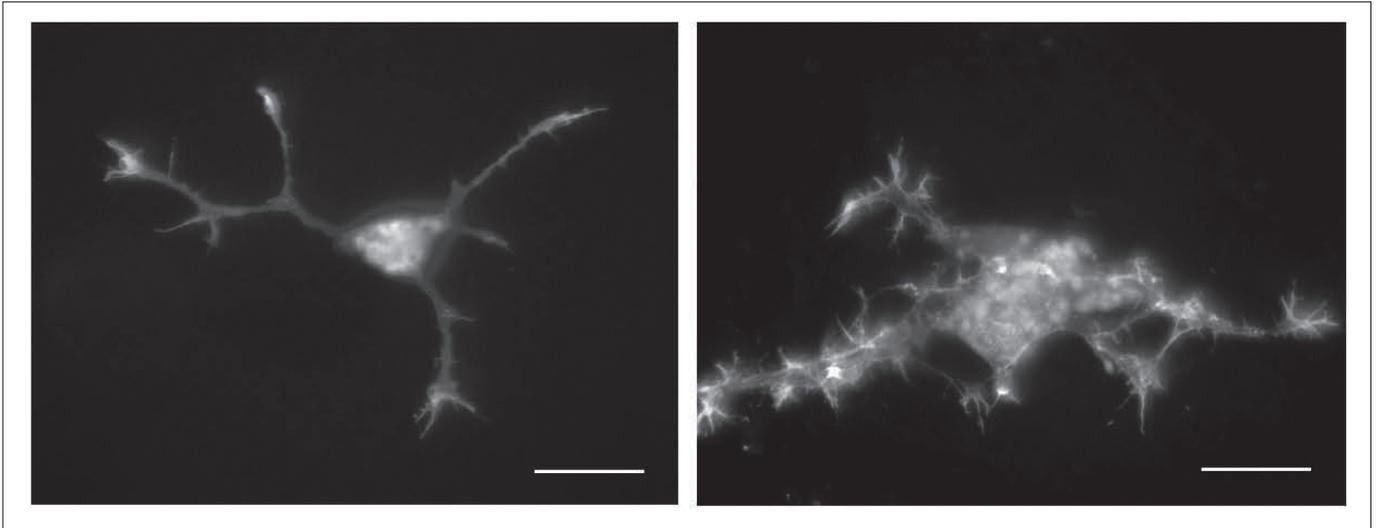
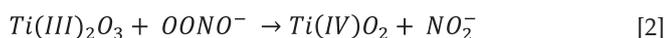
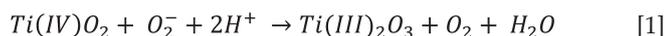


Figure 2. Fluorescence microscope photographs of *Xenopus* neuronal cell cultures over cover glass substrate (in the left) and TiO<sub>2</sub> substrate (in the right). Wider lamellipodia and numerous filopodia are observed on the TiO<sub>2</sub> substrate. Scale bar= 40 μm (40).

dioxide (TiO<sub>2</sub>) produced during the fast passivation process in presence of aqueous environment. This TiO<sub>2</sub> layer formed blocks the oxidation reaction protecting the metal from an irremediable dissolution (32). Studies have demonstrated that this TiO<sub>2</sub> nanometric film created is responsible of the high biocompatibility of the materials; it is able to neutralize free radicals produced during the inflammatory process associated to the implant procedure (33).

There are several experimental works which described this capability; however, the mechanism has not already been explained. Fenton reaction describes the decomposition of H<sub>2</sub>O<sub>2</sub> in reactive species OH<sup>·</sup>, OH<sup>-</sup> and O<sub>2</sub><sup>-</sup> and molecules H<sub>2</sub>O y O<sub>2</sub> in presence of some metallic surfaces. Tengval *et al.* (34) explain that the reaction is carried out thanks to the presence of Ti (III) in the sample. This Ti<sup>3+</sup> come from the Ti<sup>4+</sup> reduced to compensate oxygen defects in the crystal network. The decomposition runs simultaneously through the Ti-H<sub>2</sub>O<sub>2</sub> complex formation to the final product, the adduct TiOOH. The gel complex formed (TiOOH) where Ti is in oxidation state +4, tie up O<sub>2</sub><sup>-</sup> species and neutralize OH<sup>·</sup> formation reaction (34).

Two decades later, when the neutralization capability of reactive species as ONOO<sup>-</sup> by titanium oxide was proved, Suzuki *et al.* (35,36) suggest that the mechanism occurs through a redox reaction where titanium is in +3 and +4 oxidation states, which could involve possible phase changes [1] and [2]:



Redox reactions commonly happen in our metabolism and respiration. However, is well known, that TiO<sub>2</sub> reduction to Ti<sub>2</sub>O<sub>3</sub> requires high temperatures and reducing atmosphere to be accomplished (37). For this reason, while is feasible to think that the mechanism involves changes in the oxidation state, is not possible to affirm that this phase transformations could take place in the physiological conditions.

Moreover, the idea of the mechanism going through redox reactions where titanium is in +3 and +4 oxidation states

are involved suggests a relationship between the capability to neutralize radical species and the concentration of these species. The increase of Ti<sup>3+</sup> concentration in the TiO<sub>2</sub> samples, by doping or thermal treatment increases the neutralization activity of the radicals respect to un-changed TiO<sub>2</sub> (33). TiO<sub>2</sub> defects, as Ti<sup>3+</sup> formed from Ti<sup>4+</sup> to compensate oxygen vacancies, play an important role in the neutralization mechanism of radical species. According with this idea, Magnéli phases, where titanium is found in oxidation states +3 and +4, were studied on this regard and they presented an enhanced capability to neutralize the ROS/RNS involved in the inflammatory process (26). These types of materials will be studied later in detail due to their electrical and electrochemical properties to be used as electrodes in electrostimulation. Based on this idea, other materials have been suggested to induce neuroprotection. For example, nanoparticles composed of cerium and yttrium oxides can function as direct antioxidants by inhibiting ROS and RNS production pathway (38) due to the mixture of oxidation states.

Based on this capability to eliminate radical species, TiO<sub>2</sub> ceramics have been suggested as possible substrate for nervous tissue regeneration avoiding or reducing inflammatory processes, which suppose one of the main problems for the CNS repair. For this reason, regarding to the cell behavior, different studies of neurons cell cultures over TiO<sub>2</sub> bulk substrates have been already realized. First cell cultures were carried out over TiO<sub>2</sub>-rutile dense samples covered with a solution of poly-l-lysine (PLL). Results showed that TiO<sub>2</sub> as rutile phase permits good survival of mammalian CNS neurons for at least 10 days in culture (39). Neurons generally extended axons with normal appearance but they had few and short dendrites with some differences being observed in relation with the temperature of annealing of the surfaces. Furthermore, cells cultures realized straight over the TiO<sub>2</sub>-rutile substrate showed a numerous number of filopodia and branch compared with the control Fig. 2. It means a great affinity between neurons and surface material. This affinity could be promoted by the surface chemistry. In watery medium at pH 7 -OH groups are linked to the material surface, and create electric charges in the interface

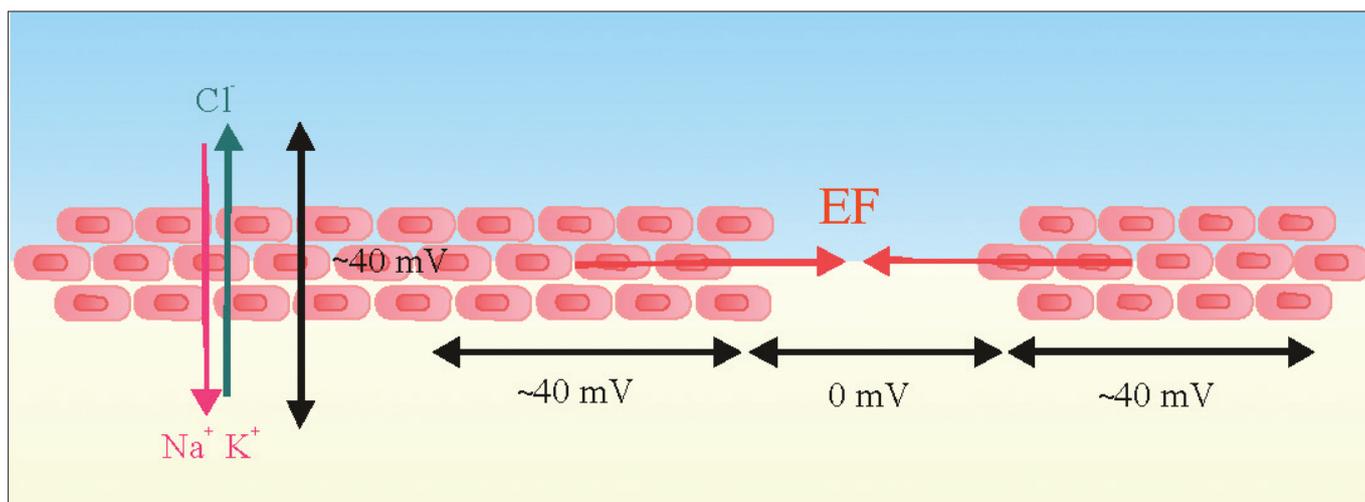


Figure 3. Mammalian corneal epithelium model.

responsible for the electrostatic surface interaction with macromolecules. Secondly, the presence of nanofeatures in the topography which are in the same size of molecules involve in the interaction. However, the main disadvantage with these substrata were the shorter axons found in cells cultured over the  $\text{TiO}_2$  substrate compared with the control; which was the result of a decrease in the energy that the cell invests in axon growth because it uses that energy in branching.

### 2.3. Electrical stimulation

The current treatment of CNS injury, particularly injuries in the spinal cord, relies on minimizing the secondary injury and implementing a physical therapy designed to help the patient with limited mobility to recover the CNS functionality. Last developments in CNS tissue regeneration provide a method for repairing functional tissue and restoring sensory and motor function (2,41).

Electric field (EF) stimulates different excitable tissues (42) but is a technique commonly used for blindness and cardiac tissue. Chemical, physical and electrical gradients coexist inside and outside cells, these gradients are known to interact; cells simultaneously read and integrate responses to both chemical and electrical cues (43). These signals are transduced to regulate cell behaviors, both in vitro and in vivo. Bioelectricity influences fundamental cellular processes as control of the cell cycle, cell proliferation, cancer-cell migration, electrical signaling in the adult brain, embryonic neuronal cell migration, axon outgrowth, spinal-cord repair, epithelial wound repair, tissue regeneration and establishment of left-right body asymmetry (44).

The mammalian corneal epithelium model is an excellent example of cells directional growth inside a physiological EF. The apical domain of each polarized cell is therefore enriched in  $\text{Na}^+$  channels and  $\text{Cl}^-$  transporters, whereas the basolateral domain contains  $\text{Na}^+\text{-K}^+$  ATPases. This polarized distribution results in net movement of  $\text{Na}^+$  and  $\text{K}^+$  into the stromal layer and net movement of  $\text{Cl}^-$  into the tear fluid. The consequent separation of charge across the tightly sealed, multilayered epithelium results in a measurable transepithelial potential (TEP) difference of  $\sim 40$  mV, with the stroma positive relative to the tear fluid. Upon injury, the TEP collapses instantaneously

to zero at the wound center, but it remains  $\sim 40$  mV distally ( $\sim 1$  mm), where ions transport is unaffected. This voltage gradient establishes an EF in the corneal tissues that has a vector parallel to the epithelial surface and the wound center as cathode (45). In addition to directing neuronal growth, the EF controls cell migration during re-epithelialization by steering cells towards the cathodal wound center (Fig. 3) (43,44,46).

Current and emerging neural prostheses and therapies based on nerve stimulation and recording electrodes include upper and lower limb prostheses for spinal cord injury and stroke; bladder prostheses; cochlear and brain-stem auditory prostheses; retinal and cortical visual prostheses; cortical recording for cognitive control of assistive devices; vague nerve stimulation for epilepsy and depression; and deep brain stimulation (DBS) for essential tremor, Parkinson's disease, epilepsy, dystonia, and depression. In most neural applications, electrical stimulation is applied as a series of biphasic current pulses (47). However, in other cases, the use of electric field (EF) in direct current (DC) has been proposed as guide for directional growth of axons and dendrites (48). The influence of the substrate surface on the direction of *Xenopus* spinal neurite growth in the presence of a DC EF at physiological magnitude was studied. In these experiments it was observed an orientated growth depending of the charge surface, getting promising results over Falcon surfaces (49).

One important disadvantage in electrical stimulation is the possibility that irreversible electrochemical reactions take place onto electrode surface or medium. The first example is water electrolysis [3] (50), with the corresponding pH change and gases generation, electrode solution by soluble metallic oxide complexes formation and oxidation of organic compounds as glucose or tyrosine (47,51).



For this reason materials used with this aim require excellent electrical and electrochemical properties apart from good biocompatibility.

#### 2.3.1 ELECTRODES IN BIOMEDICINE

Effectiveness of electrical stimulation means that current created by the electrode is translated as ions flux able to arrive

close to the tissue. The charge movement can be carried out through two different mechanisms. Capacitive charge-injection mechanism goes through a charge-discharge of the double layer electrode-electrolyte. The advantage of this mechanism is that no chemical species are created or consumed during a stimulation pulse, avoiding damages in the surface electrode or surrounding tissue. Because the double-layer charge per unit area at an electrode-electrolyte interface is small, high charge-injection capacity is only possible with capacitor electrodes with high porosity or employ high dielectric constant coatings. Some examples of this kind of electrodes are titanium nitride (TiN) (52,53). The water oxidation-reduction limits are from -0.9 to 0.9 V in this case. Moreover, there is the faradaic charge-injection mechanisms which involved the transfer of an electron across the electrode-electrolyte interface and require that some species, on the surface of the electrode or in solution, undergo a change in valence, i.e., are oxidized or reduced. It could lead to irreversible processes that cause electrode or tissue damage.

The noble metals of choice for neural stimulation are Pt and PtIr alloys (47). These metals inject charge by both faradaic reactions and double-layer charging. The relative contribution of each process depends on the current density and pulse width, although the faradaic processes predominate under most neural stimulation conditions. These types of electrodes are used for intracortical and intraspinal stimulation for their application as microelectrodes (54). With this aim, microelectrodes with high charge injection capacities have been developed. In this direction are developed hydrated Iridium oxide coatings (47,55-57). That can be obtained by two different protocols: electrochemical activation of metallic iridium (AIROF) or deposition of Iridium oxide film by sputtering (SIROF) (58). AIROF coatings, the hydrated oxide film is obtained by alternate redox reaction in succession. This increases the active surface and consequently the charge-injection capacity extraordinarily increases, and a reversible and fast faradaic mechanism takes place through a redox reaction between  $\text{Ir}^{3+}$  e  $\text{Ir}^{4+}$  species (59). SIROF films are obtained by sputtering technique in reactive Iridium oxidative plasma. The technique allows controlling charge capacity and superficial morphology by controlling the sputtering conditions (60). The degradation suffered in AIROF and SIROF coatings over the Utah microelectrodes tips was realized under electrical stimulation changing the amplitude and width of current pulses. It shows how the degradation limit in SIROF coatings was higher than AIROF, indicating the suitability of SIROF for the present application. The degradation limit in the coating depends on the charge injected and the charge density per phase which maintain a synergetic relationship. The  $\text{IrO}_x$  stability depends on the physical properties like density, roughness, thickness, as well as the pulse parameters, in case of electro stimulation by pulses. This stability is assigned to the higher density of SIROF coating compared with AIROF coating. This density is translated in a higher number of Ir ions which decrease with material polarization (58).

The geometric surface of electrode defines charge density and current parameters. A charge balance between the electrode surface and physiological medium or tissue must be maintained to avoid damages in the tissue or the electrode surface (47).

Other materials proposed with this aim are carbon nanotubes and fibers (61,62). According with their excellent

electrical properties they have been suggested as electrodes (47). Nanotubes geometry is characterized by a high ratio between the electrochemical surface area and geometric surface area (ESA/GSA). This provides the material with a high charge storage capacity through the double layer mechanism. The main disadvantage of these types of materials is the cytotoxicity, however, chemical modifications has been developed to improve their biocompatibility (63).

Pioneering work propose nanostructured hybrids  $\text{IrO}_x$ -carbon nanotubes which is a synergetic sum of the optimal properties of both components (64).  $\text{IrO}_x$  is one of the most promising materials for neural recording and stimulation electrodes because of its high electrochemical efficiency, good biostability and significant biocompatibility (64). Moreover, carbon nanotubes present exceptional low density and high superficial area in addition to desirable mechanical, thermal and conductive properties (64). Furthermore, these nanostructured composites prevent the most significant drawbacks for each as the phagocytosis in case nanotubes (64).

Nowadays, the use of new materials applied as electrodes in biomedicine is an emerging field. Conductor polymers as polyetilendioxitiofene (PEDOT) (65-66), polypirrol (PPy) or polyaniline are able to neutralize radicals as DPPH $\cdot$ , which is an indicative of biocompatibility (67).

Furthermore, polymeric-ceramic composites were previously mentioned as fruitfully candidates in biomaterials field. In case of electrodes, conductive polymers can be used as organic part of hybrid material. PPy and PEDOT could be good candidates because of his biocompatibility.  $\text{IrO}_2$  has been suggested as inorganic component due to the excellent properties already mentioned. These composites show conductivity, capacity of charge delivery and biocompatibility for growing and differentiation of neurons (68). Moreover, these work show the existence of a critical concentration of the composite (50 ng/ $\mu\text{L}$ ), from which cells were significantly affected by toxicity (68).

Piezoelectric materials have been also considered for electrical stimulation. These materials produce a transient electrical response when they are mechanically deformed (69). As a result, an electrical stimulus may be possible without direct connection to a voltage source. According with electrical stimulation here exposed, to improve neural growth few studies have investigated neural tissue engineering applications of piezoelectric materials. A study regarding to piezoelectric properties of polyvinylidene fluoride (PVDF) polymer (70) where mechanical stress is caused by vibrations was realized. The piezoelectric response enhanced neural cell (Nb2a) differentiation and neurite outgrowth compared to non-piezoelectric controls.

Ceramics, such as zinc oxide (ZnO) and silicon dioxide, both have piezoelectric properties. Though ZnO alone is not a suitable candidate, it may be mixed with a polymer to form a nanoroughness composite which is flexible and can easily be fabricated into tubular shapes. The piezoelectric response of nanomaterials may be superior compared with the piezoelectric response of a conventional material due to the large surface to volume ratio inherent to nanoparticles (71). Moreover, atoms in nanoscale ZnO structures are able to assume different positions due to the free boundary which may enhance the piezoelectric effect. Based on these ideas, several nanostructures of ZnO have been fabricated showing different piezoelectric response when mechanically deformed.

ZnO nanoparticles with approximately spherical shapes are currently the only commercially available nanostructures [Nanophase Technologies, Romeoville, Illinois], but elongated structures may be more easily deformed to produce a piezoelectric effect (2).

Some form of applied stimulus will be needed to deform the scaffold. Ultrasound could potentially be applied near the area of the implanted scaffold to provide a transcutaneous stimulus. Previous studies have shown that piezoelectric materials implanted beneath 3 cm of skin and soft tissue produced an electrical response when stimulated via external ultrasound (72). According with the information collected from studies of axon guidance under an electrical stimulus, the direction of the electric field will be controllable. This could potentially be achieved either by aligning piezoelectric nanofibers in the scaffold or applying a directional ultrasound stimulus to randomly oriented piezoelectric nanoparticles or nanofibers (2).

The next generation of biomaterials should include combinations of the known stimulatory cues. Conductive or piezoelectric nanomaterials may elicit superior regenerative response compared to conductive, piezoelectric, or nanoscale materials alone. To date, few studies have combined nanomaterials with such known stimulatory electrical cues.

Has been shown, a wide range of materials suggested as electrodes in biomedicine. Biocompatibility and capability to charge transfer from the electrode surface to the tissue are essential for this application. Moreover, the charge transfer mechanism is really important. Capacitive charge-injection mechanism which goes through a charge-discharge of the double layer electrode-electrolyte is desirable. In this way, no chemical species are created or consumed during a stimulation pulse avoiding damages in the surface electrode or surrounding tissue. TiN is one of the most representative and studied materials which possess a Capacitive charge-injection mechanism. For this reason, this material receives a special attention in this work. Finally, Magnelli phases as  $Ti_4O_7$  and  $Ti_5O_9$  have been recently studied as electrodes for this application. It is an innovative idea whereby these materials explanation is following extended and the results of this work are detailed.

### 2.3.1.1 Titanium nitride (TiN)

TiN is an example of a chemically stable metallic conductor with good biocompatibility. Charge is injected through the electrode-electrolyte double layer, and large charge-injection capacities ( $Q_{inj}$ ) are obtained by fabricating electrodes with a high surface roughness such that the ESA greatly exceeds the GSA. In many respects, porous TiN exhibits the ideal features (73,74) for intracortical capacitor electrodes, with the difficulty of obtaining high ESAs in microelectrodes of small GSA being overcome by the use of sputter deposition to produce a highly porous (sometimes called fractal) geometry. The potential limits for avoiding irreversible reactions on TiN are wider than those for Pt or Ir oxide. Water reduction and oxidation potentials measured by slow-sweep-rate cyclic voltammetry (CV) are at  $-0.9$  V and  $0.9$  V respectively (versus  $Ag|AgCl$ ). Fractal TiN, with a very high ESA, is used extensively as a coating for cardiac pacing electrodes because the electrode polarization during a pacing pulse is minimal allowing the electrode to sense the cardiac contraction in the interpulse period (75).

Porous TiN electrodes, and equivalently any electrode with a high ESA/GSA ratio, have a large charge storage capacity (CSC) as measured at low rates by slow-sweep-rate CV. However, under high rate and high current density conditions of neural stimulation pulse, the access to the whole available charge is limited by pore resistance. For all high-surface area electrodes, pore resistance imposes a geometric limitation on the increase in charge-injection capacity that can be obtained by increasing the ESA/GSA ratio. The electrolyte resistance and capacitance on the pore cavity surface combine to form a delay-line with a time-constant defined by the pore geometry, electrolyte resistivity, and the interfacial double-layer capacitance. The consequence of this time-constant is that the total ESA of the electrode is not accessed at the current densities encountered during stimulation. Narrower and deeper pores give rise to higher time-constants, and their charge-injection capacity possess a more difficult access than that of electrodes with wide and shallow pores (47).

A useful feature of TiN is the intrinsic chemical and mechanical stability of the coating. TiN is not sensitive to dehydration, can be sterilized by common methods, and is easily stored and shipped, wet or dry. Porous TiN can be patterned through photolithographically and is suitable for flexible substrates, and for this reason it is a suit candidate for intracortical silicon microprobes, retinal electrode arrays, and some nerve cuffs designs (47).

Recent work has developed TiN bulk by nitration of  $TiO_2$  at  $1100$  °C in  $NH_3$  atmosphere. These electrodes transfer charge in phosphate buffer solution (PBS) and Neurobasal media (NB) by capacitive mechanism (26). The charge storage capacitive are  $15,3$  and  $8,4$  mC/cm<sup>2</sup> respectively (26). These values are higher than the ones found in the literature for this kind of electrodes (47).

### 2.3.1.2 Magnelli phases: $Ti_4O_7$ and $Ti_5O_9$

Other material recently suggested and studied to be used as electrode in biomedicine are Ti Magnéli phases, they are substoichiometric compositions with general formula  $Ti_nO_{2n-1}$  where  $4 \leq n \leq 20$  (76). The ratio O/Ti increase from 1,75 to 2.

TABLE 1. SHOWS SOME PHYSICOCHEMICAL CHARACTERISTICS FROM THESE OXIDES:

Compound	x value in $TiO_x$	Estructure	Theoric Density
$TiO_2$	2,00	Rutile / Anatase	4,25 / 3,89
$Ti_{10}O_{19}$	1,90	---	---
$Ti_9O_{17}$	1,89	Triclínic	3,75
$Ti_8O_{15}$	1,88	Triclínic	3,84
$Ti_7O_{13}$	1,86	Triclínic	3,90
$Ti_6O_{11}$	1,83	Triclínic	4,00
$Ti_5O_9$	1,80	Triclínic	4,31
$Ti_4O_7$	1,75	Triclínic	4,32
$Ti_3O_5$	1,67	Monoclínic	4,35
$Ti_2O_3$	1,50	Tetragonal	4,59

These phases present defect structures with long range order form. The study of these phases by XRD shows that these defects structures are based on oxygen vacants ordered in plains (Fig. 4) (77, 78).

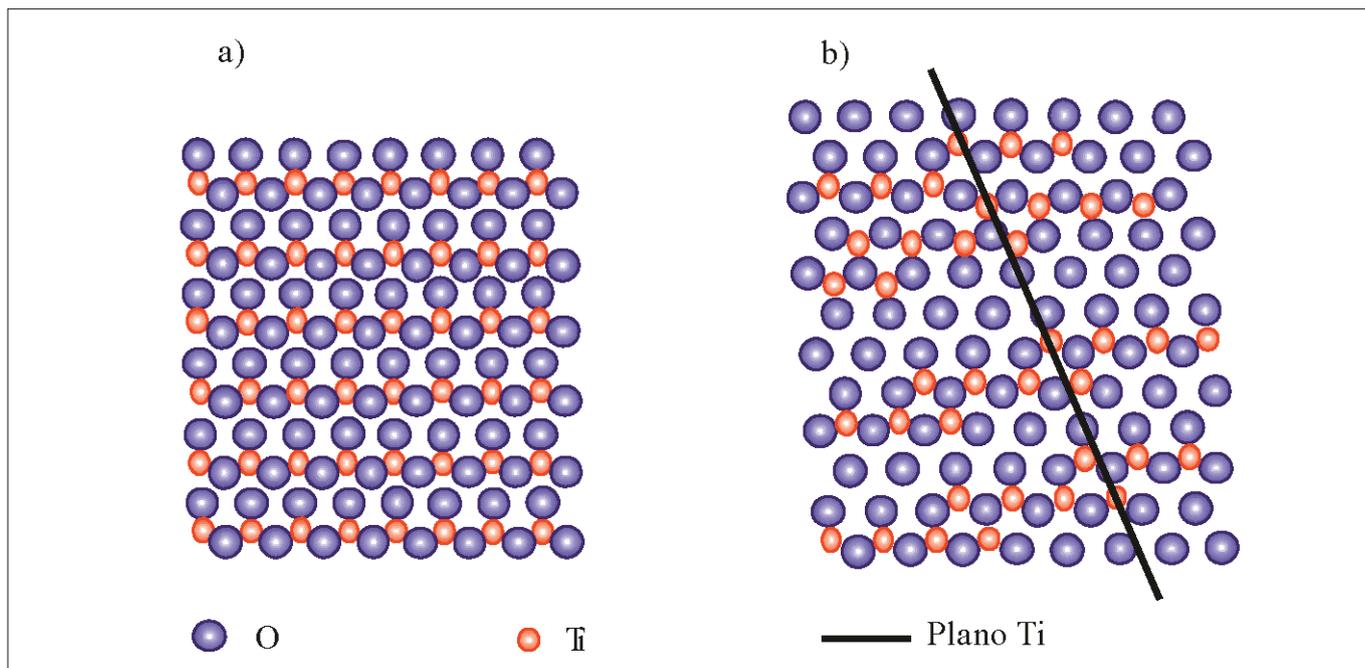


Figure 4. Atomic structures on the plain [100] from a)  $\text{TiO}_2$  ideal rutile and b)  $\text{Ti}_4\text{O}_7$ .

Based on theoretical approximations, experimental data show that at low oxygen chemical potentials, oxygen defects in enough concentration prefer to be organized in plains giving rise to Magnéli phases structures (78). Crystallographic structure can be studied from  $\text{TiO}_2$  rutile and consist in following blocks of it separated by  $\text{TiO}_6$  crystallographic plains. Rutile blocks are infinite in two dimensions and  $n$   $\text{TiO}_6$  octahedral plains in the third dimension (77).

There are several processes to reduce  $\text{TiO}_2$  like electrochemical reduction, mechanochemical or carbothermal (79-84). But the main process to obtain the Magnéli phases, is by reduction from  $\text{TiO}_2$  in thermal treatment at temperatures higher than  $1000^\circ\text{C}$  and in low-oxygen containing atmospheres, as  $\text{H}_2$  (76,85,86).

During the reduction process oxygen vacancies are compensated with  $\text{Ti}^{3+}$  formation from  $\text{Ti}^{4+}$ . So Ti is in oxidation state +3 y +4 on these materials. As previously mentioned, these  $\text{Ti}^{3+}$  and  $\text{Ti}^{4+}$  species could be involve in the scavenging mechanism of radical species related to the inflammatory response. Was demonstrated that scavenger capability of  $\text{Ti}_4\text{O}_7$  and  $\text{Ti}_5\text{O}_9$  is higher than the capability of  $\text{TiO}_2$ . It was related with the major presence of  $\text{Ti}^{3+}$  species in these materials compared with  $\text{TiO}_2$  (37).

Moreover, these materials, which has been widely studied, present interesting electrical electrochemical and magnetic properties. Ti Magnéli phases, commercially known as Ebonex<sup>®</sup>, have a high electrical conductivity (Fig. 5) at room temperature that is governed by electronic jump mechanism. Their conductivity is characterized by a conduction mechanism transition semiconductor-metal at a critical temperature between  $60^\circ$  to  $70^\circ\text{C}$ . Moreover, these type of materials present high resistance in hard redox conditions. All these properties makes than one of the most important application of these materials is as electrodes (86-90).

For all these reason, Magnéli phases as  $\text{Ti}_4\text{O}_7$  and  $\text{Ti}_5\text{O}_9$  have been recently suggested as electrodes for medical devices

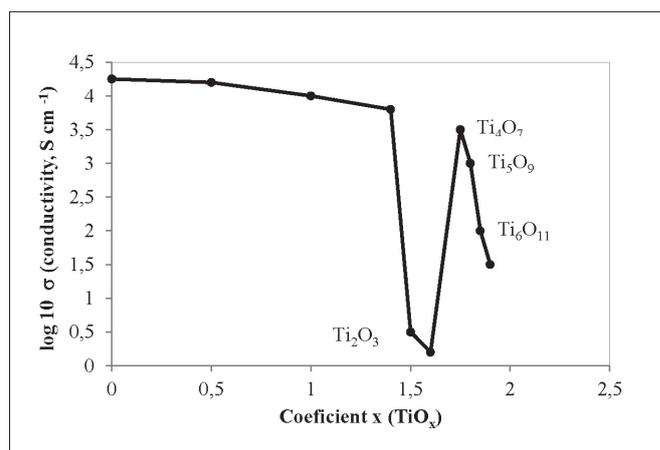


Figure 5. Electrical conductivity of different non-stoichiometric titanium oxide phases between Ti and  $\text{TiO}_2$  (89).

obtaining promising results. Materials developed from  $\text{TiO}_2$  reduction in  $\text{N}_2/\text{H}_2$  (90:10) atmosphere at  $1400^\circ\text{C}$ , showed a high porosity in the surface, high electrical conductivity at body temperature,  $>9500 \text{ S/cm}$ , electrochemical stability at physiological conditions and no toxicity (86). Moreover, transfer charge in PBS and NB is carry out through capacitive mechanism (26, 86). The charge storage capacitive are 77 and  $107 \text{ mC/cm}^2$  respectively (26,85). These values are so much higher than the ones found in the literature for electrodes with capacitive mechanism (47).

#### 2.4. Materials oriented to delivery drugs

Finally, biomaterials are applied as drug delivery systems. For example, hydrogels obtained by self-assembly (91). In CNS engineering they have two possible aims. First, materials work as cell-immobilization systems (1). Cells that secrete therapeutically active agents are enclosed by

selectively permeable membrane; it avoids the immune rejection by mimicking the cell natural medium (92). In other way, materials can be used as vehicles of neuroprotective agents, growth factors and anti-inflammatory drugs through the blood-brain barrier (BBB) (1). Approximately 98 % of small molecular weight drugs and almost 100 % of larger molecular weight peptides and proteins do not cross the BBB (93). Materials have been designed to be used as *Trojan Horse* of molecules, which are delivered after cross the mentioned barrier. Inside of this group are liposomes, biodegradable polymeric nanoparticles, polymeric micelles and dendrimers (94).

Ceramics have a place as delivery vehicles. A number of porous calcium phosphate ceramics have been developed for applications in both tissue engineering and drug delivery systems (95). Recently, ceramic nanoparticles have been developed as drug or molecule vehicles. For example, calcium phosphate nanoparticles (CaP-NP) are ideal tools for transfection due to their high biocompatibility and easy biodegradability. After transfection these particles dissociate into calcium and phosphate ions, physiological components found in every cell, and it has been shown that the small increase in intracellular calcium level does not affect cell viability (96).

In the specific case of ceramics materials directed to delivery drugs in CNS have been developed nanostructured TiO<sub>2</sub> devices. It was synthesized using a sol-gel process in order to control pore size distribution and particle with the aim to obtain a constant drug release rate for antiepileptic drugs directly into the central nervous system (CNS). Point defects were generated in the titania network in order to obtain the desired interaction between a highly polar drug and the titania device (97).

### 3. CONCLUSIONS

Ceramic materials seem to be a great solution to repair CNS injuries due to their good biocompatibility, high hardness, improved corrosion resistance, wear-resistance properties and processing possibilities. In case of materials used as substrates in CNS repair biocompatibility is essential to avoid inflammatory process which are especially painful for this kind of tissue and inhibit its normal regeneration. The processing possibilities permit to obtain porous 3D structures to allow cell colonization and nutrients arrival. For materials used as electrodes, biocompatibility avoids inflammatory process and the consequent surface electrode insolation where ions flux between material and tissue take place. Moreover, these electrode materials require corrosion and wear resistant to avoid electrode degradation and formation of damaging species for the tissue.

Between them, TiO<sub>x</sub> materials show a high biocompatibility due to their capability to neutralize radical species, which trigger the inflammatory process, thanks to the presence of Ti<sup>3+</sup> and Ti<sup>4+</sup> species. It allows controlling inflammatory responses due to the injury, and responsible of the secondary cell death, or due to the implant. Moreover, TiO<sub>2</sub> shows great affinity by neural cells which present high number of filopodia and branches over the material surface. It has been suggested as vehicle for delivery drugs for CNS repair too. In other hand, Magnelli phases as Ti<sub>4</sub>O<sub>7</sub> and Ti<sub>3</sub>O<sub>5</sub>, are excellent candidates

to be used as electrodes in CNS engineering because of their excellent electrical and electrochemical properties. They present an important capability to neutralize radical species. TiN already has been suggested for electrodes due to its electrical and electrochemical properties.

While ceramics present a great biocompatibility, promote cell attachment and healthy environment for cell growth, their mechanical properties should be improved for be implanted. At this point, the development of polymeric-ceramic composites seems to be another promising solution as substrates for CNS tissue engineering, where biocompatible and biodegradable polymers and hydrogels provide flexibility to the scaffold and can be used as drug vehicles.

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