We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Chapter

Biomaterials and Cellular Systems at the Forefront of Peripheral Nerve Regeneration

*Rui Damásio Alvites, Mariana Vieira Branquinho,
Ana Rita Caseiro, Sílvia Santos Pedrosa, Ana Lúcia Luís,
Stefano Geuna, Artur Severo Proença Varejão
and Ana Colette Maurício*

Abstract

Peripheral nerve injuries remain a common clinical complication, and currently available therapies present significant limitations, often resulting in poor and suboptimal outcomes. Despite significant developments in microsurgical approaches in the last decades, no effective treatment options have been disclosed. Current research focuses on the optimization of such microsurgical techniques and on their combination with other pro-regenerative factors, such as mesenchymal stem cells or biomaterials. Mesenchymal stem cells present a remarkable capacity for bioactive molecule production that modulates inflammatory and regenerative processes, stimulating peripheral nerve regeneration. In parallel, efforts have been directed towards the development of biomaterial nerve guidance channels and nerve conduits. These biomaterials have been optimized in terms of biodegradability, ability to release bioactive factors, incorporation of cellular agents, and internal matrix architecture (to enable cellular migration and mimic native tissue morphology and to generate and bear specific electrical activity). The current literature review presents relevant advances in the development of mesenchymal stem cell and biomaterial-based therapeutic approaches aiming at the peripheral nerve tissue regeneration in diverse lesion scenarios, also exploring the advances achieved by our research group in this field in recent years.

Keywords: peripheral nerve injuries, nerve regeneration, cell-based therapies, biomaterials, animal models

1. Introduction

Peripheral nerve injuries result in temporary or permanent interruption of the connection between the nervous system and the effector organ, a phenomena defined as denervation, leading to functional changes and, ultimately, atrophic events [1]. These injuries bear significant impact to patients' health and well-being at both functional/physiological and psychological levels [2]. The negative impact of such morbidities is not limited to human individuals, and veterinary patients

often present with comparable morbidities, contributing to the increased demand for improved therapeutic techniques [3]. Severe traumatic events (such as falls, road, or occupational accidents) result in the involvement of peripheral nerve structures in about 1–3% of the cases [3, 4]. Peripheral nerve affections may also present secondary to medical procedures such as surgeries, chemotherapy [5], and radiotherapy [6] or occur in consequence of chronic conditions such as neoplastic or metabolic diseases [7–9].

1.1 General anatomical features of the peripheral nerve

Peripheral nerve structures are composed of motor, sensory, and sympathetic fibers, forming specific nerve types that enervate the effector organs or sensory endings after emerging from the central nervous system (spinal cord) [10]. The cell bodies of sensory neurons are found in the dorsal root ganglia located in the intervertebral foramina, proximal to the site of fusion with the ventral roots. In the case of motor neurons, the cell body is situated in the central nervous system (CNS), more specifically in the anterior horns of the gray matter [11]. The nerve fiber is the functional unit of each nerve, consisting of axons and Schwann cells. Schwann cells are glial cells that are located longitudinally to the axons, of either myelinated or unmyelinated nerve fibers [10]. In the peripheral nerve, fibers of different diameters coexist, but only fibers with larger diameters are coated with a myelin sheath. In these cases, the Schwann cell wraps the axon segment concentrically. The small areas between two Schwann cells are called nodes of Ranvier, which enable the ionic exchange between the axon's axoplasm and the intercellular space and the saltatory conduction of the nerve impulse. The "jumps" of the impulses between the different nodes of Ranvier accelerate signal conduction. The fibers with smaller diameters appear grouped, and, although enveloped by the Schwann cell membrane, they are not coated by a myelin sheath. Thus, these fibers do not have the necessary structure for saltatory conduction, and the transmission of impulses along the axons is slower. Besides the myelin production, Schwann cells and their basal membrane structurally orient the axons and are sources of trophic and growth factors, ensuring the maintenance of the neighboring axons [10, 12].

The peripheral nerves are lined by three layers of connective tissue. Each axon is directly involved by the *endoneurium*, a matrix of longitudinal collagen fibers of small diameter associated with a thin network of microvessels. It grants little mechanical protection, and the capillary network acts as blood-nerve barrier [13]. Within the endoneurial layer, there are myelinated or non-myelinated fibers in association with the Schwann cells. A nervous fascicle is a set of axons covered by *endoneurium*, and the interfascicular endoneurium is the supporting framework for the nerve fibers. Nerve fasciculi may vary in number and size, depending on the nerve and the more proximal or distal anatomical position. Each nerve fascicle is further coated with a layer of consistent connective tissue called *perineurium*. *Perineurium* comprises a set of collagen fibrils of oblique, circular, and longitudinal orientation, constituted by two lamellae, working as a diffusion barrier. The outer lamella presents endocytic vesicles responsible for molecular transport. The inner lamella has tight junctions between adjacent perineurial cells, regulating the transport of macromolecules and contributing to the maintenance of the blood-nerve barrier. The *perineurium* is the greatest mechanical protection against tensile forces [14, 15]. The entire nerve trunk is further covered by a final layer of connective tissue, named *epineurium*, representing about half of the total diameter of the peripheral nerve. In some locations, the *epineurium* extends internally and separates directly the nerve fascicles (*interfascicular epineurium*). The internal portion of the

epineurium has its own network of blood vessels and varying amounts of fat tissue. The external portion, enveloping the entire nerve, defines its anatomical shape. Although the *epineurium* contributes to protection against tensile forces, it does not form specific barriers [16]. The blood supply of the peripheral nerves is achieved through the small vessels of the *epineurium*, *perineurium*, and *endoneurium*. This intrinsic blood supply system presents particular features, such as endothelial tight junctions that aid in the diffusion of compounds. Thus, this intrinsic vascular network is crucial during nerve regeneration, as the blood-nerve barrier modulates its function after the injury, allowing the flow of growth factors, immune cells, and other macromolecules into the endoneurial space [17]. The extrinsic blood supply component consists of blood vessels with different diameter that originate from larger arteries and veins in the vicinity of the nerve. Once these vessels reach the *epineurium*, they branch out, and their ascending and descending branches supply the intraneural plexuses [10].

1.2 Peripheral nerve lesions and their functional consequences

The most common type of peripheral nerve injuries are those resulting from transections (usually because of penetrating traumas), over-stretching, and compression. The effects of nerve compression are reversible when the aggression is sustained for a short period [18]. Other lesions include those caused by lacerations and ischemia [2]. Primary nerve affections originate from a force directly applied to the nerve tissue, with secondary lesions developing from the vascular and ischemic damages. Peripheral nerves have notable inherent malleability due to their collagen content. When this adaptation threshold is exceeded, the lesion occurs [19]. For example, the small arteries responsible for blood irrigation of the peripheral nerves can be compressed by hematomas developed secondarily to the initial lesion, with subsequent restriction in blood supply [20]. The main consequences are motor, sensory, and autonomic functional disturbances and deficits in the body segments that undergo denervation [21].

Seddon introduced a grading system for PNI, initially considering three levels of injury: neuropraxia, axonotmesis, and neurotmesis [22]. Later, Sunderland expanded the system to five categories, with grades 1 and 5 corresponding to neuropraxia and neurotmesis, respectively, and grades 2–4 corresponding to sub-divisions of axonotmesis [23]. Grade 4 and 5 injuries are those that require surgical intervention/reconstruction. Although this classification system correlates with the histological image of specific injury models, most lesions are mixed and encompass two or more components. In 1988 a sixth mixed injury was added to the Sunderland system by Mackinnon and Dellon (**Table 1**) [24].

Peripheral nervous system (PNS) has a superior regenerative capacity when compared to the CNS [25], due to the intrinsic and functional characteristics of each system: while the cell body of the peripheral nerve is not often affected during the lesion, CNS damage frequently results in direct neuronal death. Concerning the PNS, the age of the patient, category of the injury, and integrity of the cell body directly influence the regenerative efficiency [26]. Most importantly, the regenerative efficiency relates to the time elapsed between the occurrence of the injury and the therapeutic intervention. This time frame influences speed of recovery of nerve structures, its function, and the capacity to respond to electrophysiological stimulation, which are all essential factors in the prevention of muscular atrophy and organ dysfunctions [27].

When therapeutic or surgical interventions are delayed, the activation of Schwann cells and their stimulation over axonal growth have been demonstrated to be less effective, and more severe degenerative phenomena are observed [28, 29].

Grade	Characteristics	Functional consequences
I	• Neuropraxia • Compressive or slight crush lesions; • Schwann cell are affected, with occurrence of focal demyelination; • Wallerian degeneration rarely occurs; • The integrity and continuity of axons and connective tissue are maintained; • Occurrence of nerve conduction blocks;	• Temporary paralysis of affected body segments; • Muscle atrophy is rare; • Rearrangement of the myelin sheath within 3 to 4 weeks and recovery within days to weeks; • No need for surgical intervention;
II	• Axonotmesis; • Crushing and stretching injuries; • Disruption of the axon and its myelin coating; • Basal lamina, *Endoneurium, perineurium* and *epineurium* are intact, with maintenance of the nerve's anatomical shape; • Occurrence of Wallerian degeneration distal to the lesion site;	• Good recovery rate, the reorganization taking months but depending on the level of structural disorder and the distance to the effector organ; • Partial lesions of the *endoneurium* may occur, directly affecting the prognosis; • Rarely requires surgical intervention;
III	• Crushing and stretching injuries; • Disruptive lesion of the axon, its myelin coating and *endoneurium*; • Occurrence of Wallerian degeneration distal to the lesion site; • Intact *perineurium* and *epineurium*, with maintenance of the nerve's anatomical shape.	• Recovery can occur over several months with conservative treatment, and can be speed up if the entrapment sites that hinder the regenerative sequence are released with surgical procedures; • Misdirection of regenerating axons may occur, with development of unrecoverable functional deficits.
IV	• Crushing and stretching injuries; • Disruptive lesion of the axon, its myelin coating, endoneurium and *perineurium*; • Intact epineurium;	• Occurrence of intranerve hemorrhage and presence of fibrous tissue leads to fascicular discontinuity, with consequent imprisonment of axonal buds, delay or hindrance of its growth. • Development of neuromas in continuity is common; • Surgical intervention required;
V	• Neurotmesis; • Pungent injuries, destructive forces and local administration of toxic products; • Complete transection of the nerve, with disruption of the axons, all its coating layers and disconnection between the proximal and distal segments.	• Total loss of function; • The absence of collagen coating layers interferes with the axonal regrowth and the normal regenerative sequence; • Development of neuromas on the separated nerve stumps; • Spontaneous recovery is impossible; • Surgical intervention required;
VI	• Mixed lesion; • Common in penetrating trauma and fractures near peripheral nerves;	• Recovery and necessity of therapeutic and surgical intervention depends on the type of injury and respective degrees of severity;

Table 1.
Peripheral nerve injury grading system and respective characteristics.

Likewise, muscles and effector organs that do not receive nervous stimuli during long periods suffer more serious structural and contractile changes, and the recovery of electrical communication becomes increasingly difficult to achieve [28].

1.3 Wallerian degeneration, endogenous regeneration, and response to nerve damage

The regenerative process is preceded by an initial physiological degenerative phase [30]. Immediately after the PNI (**Figure 1a**), a complex local response is established, involving both the axon segments of the injured nerve and the surrounding non-neural cells. With no communication with the neuron's cell body, the distal axonal segment maintains the ability to transmit electrical impulses for 48–96 h after injury. However, the swelling of the axonal end occurs within few hours after injury, due to the accumulation of lysosomes and axoplasmatic organelles in the paranodal regions that cannot progress beyond the site of injury. These events incur in the stretching and thinning of adjacent myelin sheaths (**Figure 1b**). After 12–24 h, the axonal microtubules disorganize, and the dissolution of the axonal skeleton begins. In the first or second days after injury, an influx of calcium ions activates
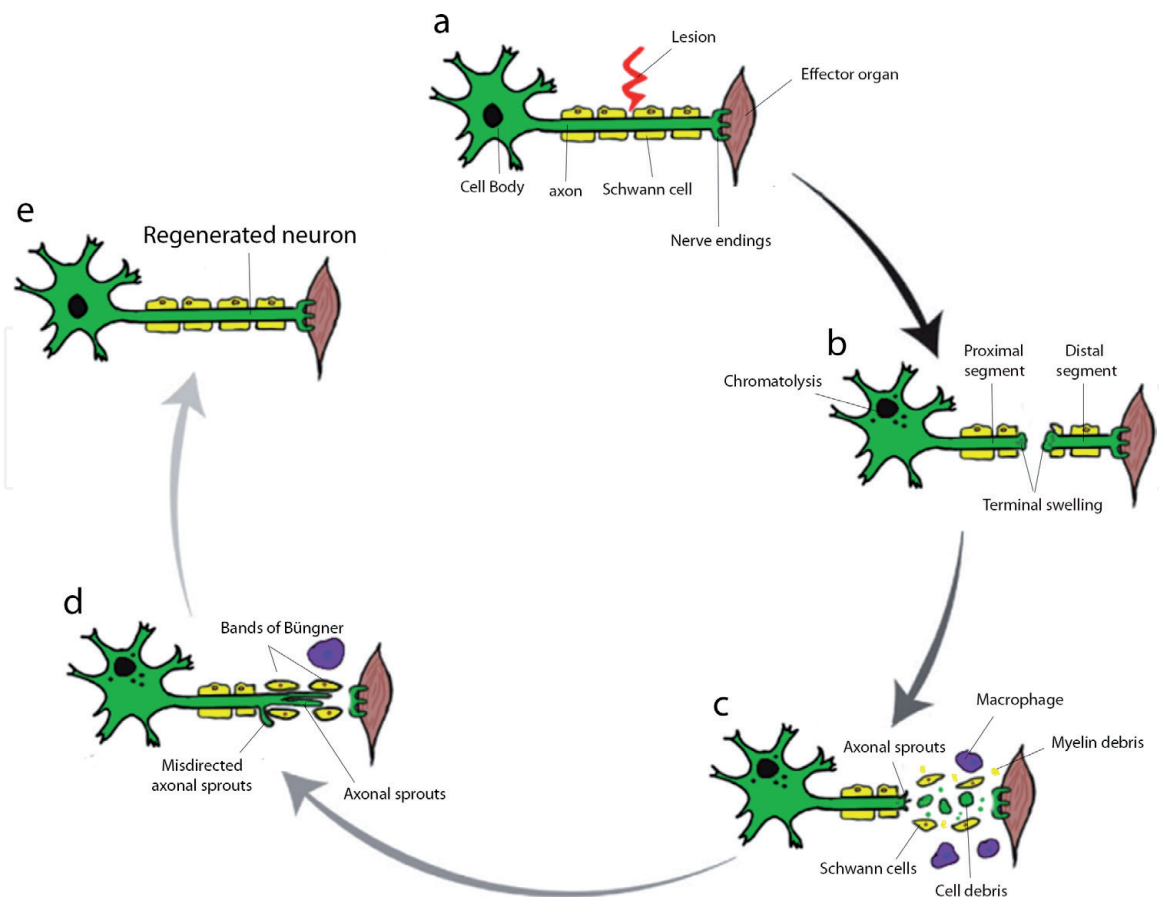
Figure 1.
Schematic representation of degeneration and regeneration of the peripheral nerve after injury: (a) PNI. (b) Chromatolysis and terminal swelling in the proximal and distal axonal segments. (c) Degradation of axons and myelin with release of debris. Cell and myelin debris are phagocytosed by macrophages and Schwann cells. Axonal sprouts are formed. (d) The axonal sprouts grow with the support of the bands of Büngner. The remaining macrophages undergo apoptosis or return to circulation. (e) Regenerated neuron.

calcium-dependent calpain proteins, which degrade axonal neurofilaments, releasing granular debris (granular disintegration) (**Figure 1c**) [31].

Within few minutes of lesion, the Schwann cells promote the degeneration of the myelin sheaths associated with the distal segment. After 24–36 h, changes become evident throughout the distal segment, with swelling of Schwann cells, compressing the associated axons. Myelin destruction is installed within 48 h after injury, which is fragmented into ovoid structures by the digestive chambers present in the cytoplasm of Schwann cells. An identical process is observed on unmyelinated Schwann cells, minus the fragmentation of the myelin [32]. Axonal and myelin fragments are then eliminated by cells with phagocytic capacity (macrophages and other myelomonocytic cells) and by the Schwann cells themselves (**Figure 1c**) [33]. The production and release of interleukins by these cells activates and stimulates the activity of other Schwann cells and fibroblastic populations [34, 35]. Once clearance of all axonal and myelin debris occurs, macrophages are eliminated by local apoptosis or by reentering in circulation.

Simultaneously, the mitotically quiescent Schwann cells are activated and proliferate within their original basal lamina, organizing themselves to create longitudinally oriented structures. These structures are called bands of Büngner and are important in the next phase of nerve regeneration, providing biochemical and structural support to the new axonal sprouts as they proliferate (**Figure 1d**) [36].

After the degeneration phase, the regeneration begins, which can be successfully concluded or abortive. The proximal segment undergoes an initial degeneration phase up to the last preserved internode in an identical manner to

that occurring in the distal segment but of smaller extension. The most important phenomenon of the proximal segment is chromatolysis, while genetic upregulation and downregulation are established, metabolically preparing cells for the next phase (**Figure 1b**) [35, 37].

In the first 24 h after the injury, many axonal sprouts protrude from the most distal node of Ranvier of the proximal segment and bulge into a growth cone rich in metabolically active organelles. The progression of these sprouts is guided by the bands of Büngner [38], which are indispensable for guiding the expanding axons and secrete neurotrophic and transcription factors, creating a conducive environment to the growth of axonal buds [29]. Proteases are also released from the growth cone to degrade the fibrous, hemorrhagic, or inflammatory tissues and facilitate the progression of the axonal sprouts [39]. Only a few of the axonal extensions will contact the receptor at the distal ends (**Figure 1d**). In theory, an increased number of axonal sprouts reaching the target segment correspond to more extensive and effective neural regeneration (**Figure 1e**). The axonal extensions that do not reach the distal segment are eliminated to prevent misdirected and disorganized growth and possible development of neuromas. Nevertheless, occurrence of misdirected or erroneous axonal growth and its subsequent ineffective innervation of target tissues is often observed [40].

The quality and speed of nerve regeneration is improved when occurring in a well-vascularized site and in the presence of small amounts of scar tissue. Besides mechanical factors, the time elapsed between injury and complete repair and its functional recuperation prognosis depend on factors such as the age of the patient, the type of nerve, the site of the nerve that was injured, the cause of the injury and effect on neighboring tissues resulting from the injury [1, 2, 41, 42]. Nonetheless, even when endogenous repair mechanisms are effective, regenerated nerves often present thinner myelin sheets and shorter nodal lengths and result in functional deficits [43].

1.4 Therapeutic options for peripheral nerve injury

There are several therapeutic options available to address PNI, ranging from conservative to surgical approaches. Despite all efforts and advances achieved in recent years towards the effective repair of peripheral nerve after injury, the ultimate outcomes are still far from ideal, and recovery rates remain limited.

The success of any therapy prospected for the application in PNI will depend on the acceleration of the axonal regeneration rate achieved and modulation of the local microenvironment, therefore impacting on the chronicity of installed denervation and established consequences in the effector organs [44]. Thus, even if initial immobilization with physical therapy may be considered in some cases to ensure patient comfort, quick surgical reconstruction, associated or not to other therapeutic options, should be favored to promote improved nerve regeneration (**Figure 2**).

The primary repair of the injured nerve resourcing to microsurgical techniques is the recommended approach in case of neurotmesis lesion. To get the desired results, however, the expected success in peripheral nerve structure and function depends on the timing of reconstruction, and the intervention must be performed within a short period after the occurrence of the lesion. Epineural repair is one of such techniques, employed when tension-free coaptation of the nerve margins is possible within a well-vascularized local microenvironment [45]. In the first phase of the surgical technique, the injured nerve endings are intervened to remove necrotic tissue, cells, and inflammatory debris (originating from neurolysis), exposing the viable nerve stumps. In the subsequent phase, the two nerve tops
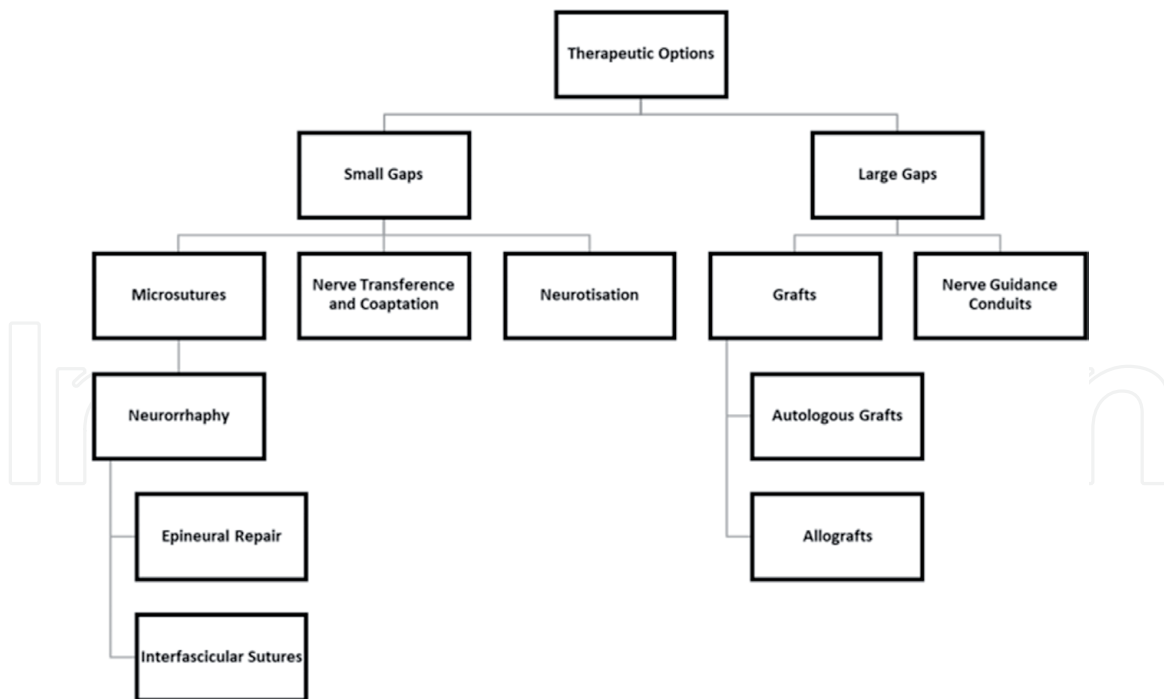
Figure 2.
Schematic representation of therapeutic options for PNI treatment.

are re-approached and anatomically coapted to achieve a minimum gap between them. This small space is quickly filled in with blood, phagocytes, and a fibrin matrix and plays a critical role in the transportation of Schwann cells between the two segments of injured nerves. Perfect coaptation between the two nerve ends is achieved through the application of interrupted micro sutures (neurorrhaphy) in the *epineurium*, aiming at the maintenance of the physiological position of the nerve segments, avoiding relative rotation displacement [46]. In larger nerve structures, the application of a micro suture between nerve fascicles of the two nerve segments after a careful intranerve dissection may be necessary. Theoretically, this technique sustains better fascicular alignment, but the trauma resulting from the extended dissection and the exuberant scarring phenomena resulting from the presence of the suture embedded in the nerve structure entail undesired side effects, precluding the success of this nerve reconstruction technique [47]. When direct neurorrhaphy is not possible [48, 49], an alternative technique includes the connection between the proximal segment of a nearby healthy nerve and the distal segment of the injured one (neurotization). A reimplantation when nerve root avulsion occurs is also possible [50].

When the injury results in nerve tissue loss and a gap forms between the two nerve segments, grafting techniques may be used to bridge the tissue gap and guide the regenerating axons in a tension-free manner [51]. Nevertheless, grafting techniques are not devoid of disadvantages [51, 52]. Autologous nerve grafts are one of the described options, due to the microstructural composition that facilitates axonal migration and the decreased risk of immune rejection reactions at the grafting site. However, this technique requires the sacrifice of a healthy nerve with proper dimensions and diameter, which is a limiting factor to its widespread application [53]. Allogeneic grafts are a complementary option, generally collected from cadaveric donors. Despite retaining the tissular microstructures, the risk of rejection at the receptor site increases, and concomitant immunosuppressive treatments are required [54]. These allografts can be decellularized through enzymatic treatment, minimizing the risk of inflammatory reaction and reducing the necessity for immunosuppression, improving their success rate [55].

7

Nerve sheets have also been used to promote the reparation of diverse injured tissues and in the promotion of nerve recovery after PNI, stimulating an early restitution of vascularization. The nerve sheet plays a scaffold function without promoting immunogenic reactions, ensuring cytokines and growth factors that stimulate axonal survival and regrowth [56, 57].

The use of nerve guidance conduits (NGCs), in a technique known as entubulation or tubulization, presents as an alternative to the use of grafts. These conduits provide physical separation between the regenerative site and the neighboring reactive (fibrous) tissue, preserving the neurogenic factors secreted by the injured nerve terminations [58–60]. The NGCs are described to outperform organic grafts in small nerve reconstruction [55], but the general lack of internal microstructural characteristics limits their application in the bridging of small nerve gaps [61, 62], particularly when not combined with cell-based therapies or locally applied growth factors (**Figure 3**). Therefore, NGCs function mostly as structural guides to promote the alignment of the two nerve ends, while the repair process of the nerve gap itself is promoted by the complementary therapies applied [63]. The biomaterials deemed adequate to shape the NGCs are expected to comply with a set of criteria that guarantee their safety and efficacy, as listed:

- Biocompatibility with target tissue (not triggering local or systemic inflammatory or organic rejection responses) [64]

- Absence of toxicity

- Biodegradability [65]

- Mechanical and structural stability during the regenerative process [66]

- Mechanical resistance to application of sutures and to the occurrence of mild local inflammatory reactions [66]

- Balanced flexibility and resistance to avoid compression of the nervous tissue during the reparation and to attenuate the hoarding of fibrous tissue and inflammatory secretions within the NGCs [67]

- Selective porosity and permeability (avoiding excessive loss of neurotrophic factors, tolerating entry of nutrients and oxygen, while controlling inflammatory cell influx to the injury site) [59, 68]

- Capacity to direct axonal growth distally [69]

- Adequate technical production, sterilizations, storing, and manipulation procedures [70]

The selection of the most suited biomaterial must also address the required dimensions and wall thickness required for the proper alignment and connection between the two nerve ends, without tension or compression, factors that may influence the rate of regeneration [71]. Diverse materials have been described, from natural and synthetic resorbable to non-resorbable devices. Regardless of the selected option, the ideal biomaterial must be capable of protecting the nerve tissue, avoid the development of neuromas, diminish the occurrence of tissue adhesions, guarantee a negligible inflammatory response, and stimulate regeneration of the axon [72].
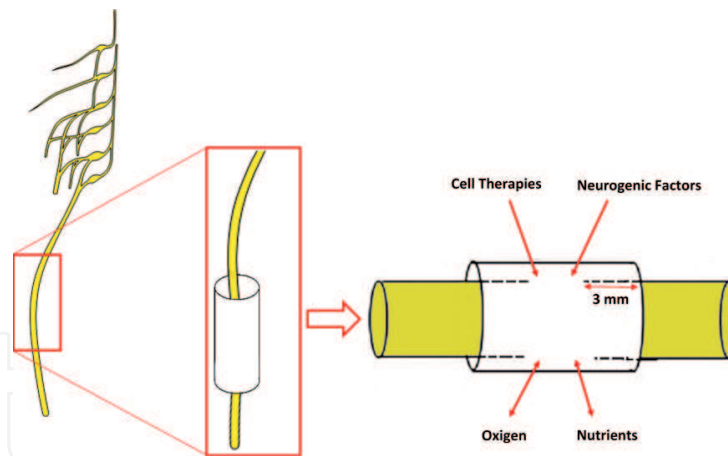
Figure 3.
Schematic representation of the application of an NGC in the rat sciatic nerve.

Although biomaterials alone can withstand, guide, and, in some extent, reestablish the continuity of the injured axons, the effectiveness of this entire process remains globally limited, particularly if longer gaps are considered [62]. In the current therapeutic scenario, the true advantages of using biomaterials reside in their combined use with cellular systems and neurotrophic factors [73]. Neurotrophic factors or neutrophins are produced and released naturally during the nerve regeneration process. These can be secreted by neuronal or non-neuronal cells, at both nerve ends, and are essential to conduct the regenerative sequence. Its function is mainly to stimulate neural differentiation and guide axonal growth [74]. Although these factors can be directly administered to the injured nerve, their application inside the lumen or on the wall of the NGCs (allowing a continuous release by diffusion into the lesion) presents as a more effective technique in longer nerve gaps. Without NGC support, the neurotrophic factors may diffuse freely towards neighboring tissues, deviating from the injury site, failing to support the regenerative process [57]. Different neurogenic factors have been proposed in studies on axonal regeneration, including glial cell-derived neurotrophic factor (GDNF); neuregulin-1, superfamily growth factor beta; brain-derived neurotrophic factor (BDNF); neurotrophins 3, 4, and 5; insulin-like growth factors; nerve growth factor (NGF); ciliary neurotrophic factor; and a combination of several factors such as platelet-rich plasma [75, 76].

Regenerative therapies based on the use of cellular agents are a promise to improve the therapeutic efficacy of techniques developed to stimulate peripheral nerve regeneration. Embryonic stem cells are theoretically the most versatile regenerative population, but their affective applicability for clinical purposes remains a contradictory topic, mostly due to the extreme ethical issues associated [77]. In the regenerative processes, native to the peripheral nerve, the Schwann cells represent a crucial regulatory role and therefore have been proposed as regenerative enablers. However, important limitations envisioning their clinical application have been noted, such as the associated donor site morbidity, the challenging ex vivo culturing and expansion protocols, and the complex therapeutic application. In addition, Schwann cells' availability is age-dependent (decreasing with the increasing age of the donor) [78].

In recent years, interest in the use of mesenchymal stem cells (MSCs) has increased significantly, particularly because of their assigned aptitude for cell and tissue differentiation and their capacity to adapt to each site of injury and to produce growth factors related to reparative phenomena. Also, they locally and systemically modulate the inflammatory reactions [79]. MSCs can be isolated

from virtually all tissues on the organism (niches), such as the dental pulp, the synovial membrane, the olfactory mucosa, the placenta, and the umbilical cord Wharton's jelly [80–83]. To be classified as "true" MSCs, these cells have to manifest a set of specific characteristics: plastic adherence under standard culture conditions; lack of expression of hematopoietic markers (CD11b, CD14, CD34, CD45, CD79α, or CD19) as well as major histocompatibility complex II/human leukocyte antigen-DR; expression of the unspecific markers CD44, CD73, CD90, CD105; and capacity to, at least, trilineage in vitro differentiation (osteogenic, chondrogenic, and adipogenic) [84]. The ease of expansion of these populations, their capacity of differentiation in different cell lines, the tropism for lesion sites, their immunoprivileged phenotype, and capacity of trophic stimulation and modulation of tissue functions turn them into excellent candidates for therapeutic adjuvants [85]. Regarding the immunoprivileges of MSCs, these cells have long been referred as hypoimmunogenic, with the ability to cross most of the histocompatibility barriers without triggering an immune response [86]. However, some studies identified the production of antibodies against these populations and immune rejection after allogeneic donation, raising debate on this topic [87]. MSCs administered from allogeneic donations have been described to promote infiltration of macrophages and neutrophils at the injection site [88, 89] and to stimulate a donor- and dose-dependent blood-mediated inflammatory reaction [90]. There are also references to adverse clinical reactions following intra-articular administration of allogenic MSCs in the equine model [91, 92] and evidence of early death of MSCs after administration [93]. To fully understand the potential and limitations of allogeneic MSC therapies, deeper investigation is required on the immune response elicited and whether such responses may affect the therapeutic outcome.

In specific PNI scenarios, MSCs are described to intervene through several mechanisms, namely, secreting neurotrophic factors that stimulate neurogenesis and proliferation of Schwann cells; undergoing neurogenic or neuroglial (Schwann cells) differentiation; or modulating the local inflammatory response and the Wallerian degeneration [94]. The neurotrophic factors produced and secreted by MSCs include ciliary growth factors, neurotrophins, endothelial growth factors, glial-derived neurotrophic factors, NGF, and BDNF [29]. Remyelination of injured axons is also promoted by the MSCs since they can differentiate into cells presenting morphology and phenotypical markers of Schwann cells, becoming capable of promoting myelination [85, 95, 96].

Despite the prospected advantages, the optimum mode of administration of MSCs to the lesion site is still unclear. The simplest technique would be the microinjection of a cellular suspension at the injury site, but associated risks include trauma to resident cells and intraneural architecture and uneven cell distribution. The individual application of the MSCs, although being associated with improved outcomes, still denotes limited advantage over the traditional surgical techniques [97]. An alternative to the direct injection of the MSCs is their combination with a supporting matrix (such as fibrin) and their combined deposition at the lesion site [98]. Overall, the efficacy of MSCs is suggested to increase when in association with biomaterials (such as NGCs) and growth factors, granting increased success rate and functional recovery [99].

2. Biomaterials and peripheral nerve regeneration

Diverse biomaterials have been proposed in attempt to establish the best options to promote peripheral nerve regeneration after PNI.

2.1 Biological nerve conduits

Natural polymers, usually based on carbohydrates or proteins, are frequently applied as scaffolds to promote the regeneration of different tissues. Natural or biological biomaterials are described to easily stimulate cell adhesion, migration, proliferation, and growth. However, batch-to-batch variations of raw materials are often observed and limit the standardization of final product composition and manufacturing protocols [100].

2.1.1 Collagen

Collagen is seen by many researchers as the "perfect" material for regenerative medicine applications, since it is a major protein constituent in the extracellular matrix. Collagen has a regenerative effect on nerve tissue by transducing essential signals that stimulate cell adhesion, migration, proliferation, survival, and differentiation. Besides, it creates a supportive environment for connective tissues surrounding the blood vessels, ligaments, bone, cartilage, tendons, and skin, in both natural environment and regeneration sites. Collagen can be found and isolated from various animal tissue sources.

The advantageous characteristics of collagen include its physical resistance, low level of antigenicity, good biological compatibility, and the ability to be tailored and cross-linked, modulating water uptake and mechanical degradation rates. Of the 27 different types of collagen identified to date, the most abundant and widely investigated in biomedical engineering and regenerative medicine is the type I [101], which constitutes approximately 30% of mammalian musculoskeletal tissue [102]. For nerve regeneration, collagen type IV, a non-fibril forming collagen and the main component of the basement membrane, must also be considered due to its interaction with the Schwann cells [103]. Studies on the effectiveness of equine collagen type III have also been performed [104].

Collagen has been utilized as a scaffold, in the form of a gelatinous matrix, to stimulate neural regeneration, but it can also be processed in different formats of three-dimensional structures like gels, porous sponges, sheets [101], particles, and foams [105]. The application of collagen filaments (not organized in a conduit shape) promoted an effective guided axonal regeneration of 20 mm nerve defects [106], and NGCs containing a gel of collagen with Schwann cells were described to induce the growing of new neurites [107]. Collagen fibers within gels can be longitudinally aligned using magnetic fields and result in an improved neurite outgrowth when compared to that observed with randomly oriented collagen fibers [108]. Moreover, collagen NGCs containing a porous collagen glycosaminoglycan matrix promoted regeneration levels similar to those resulting from nerve autograft [109, 110]. Finally, commercial collagen type III membranes (commercially available as GentaFleece®, Baxter, Nuremberg, Germany) were demonstrated to promote the regeneration of rat sciatic nerves undergoing neurotmesis [104] and axonotmesis [111].

Various collagen-based conduits/devices are FDA-approved. In the process of manufacturing these NGCs, the matrix is molded tubularly while preserving the natural fibrillar characteristics of the collagen:

- NeuraGen® (approved in 2001) was demonstrated not to cause neuropathy by compression [112], a common observation when using rigid materials, and sustained nerve repair in the period of 4 weeks [110].

- NeuroMatrix™ and Neuroflex™ (both approved in 2001; Collagen Matrix, Inc., Franklin Lakes, NJ, USA) are flexible, resorbable, and non-friable NGCs that

present a semipermeable tubular matrix, holding pores with diameters between 0.1 and 0.5 μm, thus allowing the transference of nutrients. Both nerve conduits are absorbed within 4–8 months after implantation, differ in the kink resistance, and are indicated for application in nerve defects smaller than 2.5 cm [68].

• NeuraWrap™ (2004; Integra LifeSciences Co), presenting the same constitution of NeuraGen®, is described to promote minimal scar tissue formation due to the porous outer membrane, capable of resisting compression by the neighboring tissue. Also, NeuraWrap™ promotes minimal nerve encapsulation and entrapment and avoids the formation of neuromas. Finally, it promotes an environment conducive to regeneration, because of a semipermeable inner membrane that allows the exchange and transport of nutrients [113].

• NeuroMend™ (2006; Collagen Matrix, Inc.) is a semipermeable device, designed so it can be unwound and spontaneously curl around the injured nerve, adapting perfectly to its shape and dimensions. The semipermeable membrane also allows the circulation of nutrients, regulating the movement of fibroblasts and inflammatory elements [114, 115].

Besides the available commercial nerve conduits, other studies describe the utilization of pure or blended collagen devices. Nerve conduits releasing neurotrophic factors such as GDNF and NGF were reported to result in improved reparation of nerve defects when compared with commercially available products such as Neurolac™ or NeuraGen® [57]. Micro-patterned tubular collagen matrices applied to the rat sciatic nerve model also demonstrated good pro-regenerative capacity [116]. In a work presented by Yang and Chen, the interaction between composite scaffolds obtained from blending a cross-linking chitosan with icariin and collagen revealed successful cell attachment, establishing the material as suitable for the support of cells in nerve regenerative therapies [117]. Cerri et al. compared the efficacy of collagen scaffolds with different porous microstructures in promoting sciatic nerve regeneration in the rat model of axonotmesis. A complete replacement of the conduit by normal nerve tissue was observed 60 days after injury, associated with a progressive regulation of genes and myelination, interaction between axons and Schwann cells, and angiogenesis [118].

2.1.2 Chitosan

Chitin is a biopolymer present in the shells of crustaceans and cuticle of and exoskeleton of arthropods [119]. Chitosan can be obtained from the chitin, by a process of partial deacetylation [120] commercially available by alkaline hydrolysis [121], and can be found in nature in some fungi [122]. Because of its characteristics, chitin, chitosan, and its complexes have already been explored for different medical and industrial applications, namely, for wound treatment, drug delivery systems, and space-filling implants. These biomaterials present positive features such as good biocompatibility and biodegradability; nontoxic character; low price; possibility of being modified chemically and enzymatically; antimicrobial properties; controlled release of components such as cytokines, antibiotics, and extracellular matrix components; promotion of cell adhesion; and maintenance of cell and tissue viabilities. These can also be shaped to create different forms, from films, sponges, or fibers to hydrogels and complex scaffolds [123, 124]. The described features turn these materials into adequate options for peripheral nerve reconstruction [125], with their potential to promote nerve regeneration demonstrated in vivo and

in vitro [63]. One of the main disadvantages assigned to the use of chitosan matrices is its reduced mechanical strength when exposed to physiological conditions, failing to preserve its initial shape after implantation [126].

Chitosan conduits modified with different biodegradable polymers have been developed and evaluated.

Cheng et al. tested chitosan-poly(ʟ-lysine) composite films, considering that the hydrophilic nature of chitosan appears to be essential to prevent the development of glial scars and promote nerve regeneration, observing enhanced cellular affinity and outcomes when compared to collagen films [127]. Similarly, the use of gelatin mixed with chitosan composite films displayed increased elasticity of the conduits and greater nerve cell affinity, besides promoting cell differentiation [128]. Wang et al. was able to improve nerve reparation along a large gap in the sciatic nerve of dogs using chitosan-polyglycolic acid (PGA) grafts, noting the reestablishment of nerve continuity and functional recovery [129].

Chitosan NGCs modified with inorganic components were also explored. In a study by Gärtner et al., chitosan tubes modified with apatite were used to improve their mechanical strength and avoid swelling. The application in the rat model of axonotmesis allowed the observation of neovascularization and macrophages phagocyting cell debris, thus demonstrating functional Wallerian degeneration [130].

Itoh et al. studied the efficacy of chitosan tubes obtained from tendons of the *Macrocheira kaempferi* crab in the rat sciatic nerve submitted to neurotmesis. Some tubes also had laminin and laminin peptides adsorbed, to favor the adhesion of Schwann cells and the growth produced by the neurites. At weeks 2–4 post implantation, the tubes revealed inflammatory and macrophagic infiltration due to some fragmentation of the tube wall, with evidence of peripheral nerve regeneration after 6 weeks. In tubes enriched with laminin and laminin peptides, the regenerative process occurred over the internal wall, while in the other tube formulation, it was observed inside the lumen. Nociceptive function recovery was smaller than that observed with the use of isografts [131]. Wang et al. also confirmed that the use of chitosan laminin-peptide-treated tubes resulted in an increased number of regenerated axons, many of them adhering to the inner layer of the wall, that is, the one where these substances had been covalently bound with a nano-/microfiber mesh [125].

The use of chitosan in the form of nanocomposite, in association with gold, was also described. These nanoparticles improved the mechanical force of chitosan and stimulated the proliferation of neural cells and gene expression, thus resulting in better and faster functional recovery in a neurotmesis model, with more myelinated axons than the use of the isolated composite [132].

Wang created a nonwoven nano-/microfiber mesh tube comprising on an inner layer of a nano-/microfiber chitosan mesh and an outer layer of chitosan film. It was then used in the treatment of a rat sciatic nerve undergoing neurotmesis, comparing with simple chitosan nano-/microfiber mesh tubes and chitosan film tubes. The results showed that the chitosan nano-/microfiber mesh tubes displayed mechanical properties capable of preserving the tube space, guaranteeing a good scaffold for migration and cell adhesion, and facilitated the humoral flow that stimulates regeneration [123].

The importance of the acetylation of chitosan in promoting nerve regeneration was also identified by other authors. Freier et al. compared the compressive force of chitin gel tubes and chitosan tubes with different degrees of acetylation and concluded that the lesser the acetylation rate, the superior the mechanical strength, lower the degradation rate, and greater the adhesion and viability of the cells applied. The main factor in determining cell compatibility with chitosan was

its charge, depending on the availability of amine groups. Since for lower degrees of acetylation there is an increase in charge density, it results in increased cell adhesion [121, 122]. In the works of Haastert-Talini et al. and Gonzalez-Perez et al., it was determined that the optimal degree of acetylation to stimulate nerve reparation is about 5%. Acetylation values around 2% cannot guarantee axonal regeneration, whereas acetylation around 20% degrades too early and has poor mechanical stability [133, 134].

The benefits of using chitosan may be improved by the modification of its properties but also by the concomitant use of other therapies such as growth factors or MSCs. The combined approaches aim to speed up the regenerative process and to decrease the secondary effects related to the degradation of chitosan. The chitosan polymer and its short chains do not cause an inflammatory response [135], but the fragments of its degradation can cause inflammation, leading to apoptosis of the regenerating cells and proliferation of fibrous tissue around the NGCs [119].

In the works of Patel et al., chitosan tubes enriched with laminin and GDNF were used [136]. GDNF is a trophic factor that promotes axonal regeneration, prevents atrophy of motor neurons, and relieves neuropathic pain [137]. Using this combination, nerves with a neurotmesis injury presented superior functional recovery to those submitted to unblended chitosan tubes. These results corroborate that, although they can promote nerve repair alone, isolated chitosan tubes have limited potential for regenerative promotion. Hsu et al. tested different tubes to promote nerve regeneration, comparing simple silicone tubes, laminin (LN)-modified chitosan scaffold in silicone conduit, and laminin (LN)-modified chitosan scaffold with bone marrow MSCs (BMSCs) combined with silicone conduit. The laminin (LN)-modified chitosan scaffold in silicone tubes was surrounded by macrophagic and eosinophilic hyperplasia granulation tissue after the experimental period, which was not observed in the presence of MSCs. This indicates that MSCs not only prevent the death of neurons and stimulate nerve regeneration but also reduce the inflammation and fibrotic development that may eventually be triggered by the long-term implantation of chitosan [138].

Lauto et al. tested the use of chitosan in PNI cases in a distinct perspective, developing an adhesive formula comprising chitosan, indocyanine green, acetic acid, and water. Applied in promoting rat tibial nerve regeneration, this alternative method promoted superior nerve repair, allowing connections between nerve ends stronger than those achieved with the use of fibrin glue. This chitosan adhesive presents several advantages when compared to traditional fibrin glue, namely, its insolubility in physiological fluids, its hydrophilicity, and presenting adhesiveness before laser activation [139].

The mechanical properties of the chitosan can be improved by an adjustment with a silane agent such as γ-glycidoxypropyltrimethoxysilane (GPTMS), improving its mechanical strength by promoting the wettability of chitosan surfaces. Some works have confirmed that integrating silicates into the chitosan membrane honed their cytocompatibility, making the combinations good candidates to be applied clinically [140–143]. Following these works, Amado et al. applied porous chitosan GPTMS membranes with about 110 μm pores and 90% of porosity in rat sciatic nerve injuries to study its effect on nerve regeneration [144]. The results showed that using porous hybrid chitosan membranes promoted a significantly better nerve fiber regeneration than solid membranes. The porous membranes, with a greater surface-to-volume ratio, showed the capacity to maintain mechanical strength [145] and the ability to adapt to different shapes. Its use allows an adequate revascularization of the regenerating tissue, reestablishment of metabolic communication with the surrounding microenvironment, and maintenance of nutrient and oxygen exchanges at adequate levels [146]. These conditions promote the proliferation of

Schwann cells, neurite extension, and remyelination, leading to an increased number of axons and nerve fibers and even increased thickness of myelin sheath [144].

Simões et al. also compared the use of solid and porous membranes in sciatic nerve axonotmesis and neurotmesis models, having observed a significant infiltration of multinucleated giant cells and some mast cells into the porous membrane and the development of an inflammatory reaction capsule with the solid membrane. These differences in the established inflammatory reactions may therefore justify the improved regeneration observed with the use of the porous membrane. Since the membranes with pores present a bigger surface-to-volume ratio, a higher contact with the immune system of the host can justify the greater infiltration of cells [147]. Simões et al. further compared the use of lacquered-poly(lactide-co-glycolic) acid test tubes with acetic acid and glycolic acid in a ratio of 90:10 (PLGA 90:10) with the use of chitosan porous membranes in the neurotmesis model through different surgical methods. The results revealed that although nerve regeneration occurred in all groups of animals, those with tubulization with chitosan presented better nerve regeneration and functional recovery than those receiving PLGA tubes [63]. A similar study was performed by Shirosaki et al., and the results also revealed that, although nerve regeneration was achieved in all experimental groups, chitosan porous hybrid tubulization was the treatment that promoted better nerve regeneration and functional recovery [141]. The ability of the porous chitosan GPTMS to promote nerve regeneration is probably related to its ability to promote the expression of genes related to myelin and the ions of silica that stimulate the expression of different glycoproteins [148]. This allows the nerve fibers to regenerate along the chitosan structure, establishing an extensive perineural connective architecture that ensures axonal fasciculation [63].

In June 2014, a commercial product was launched consisting of a chitosan-based nerve conduit with the name Reaxon® Nerve Guide, manufactured by Medovent GmbH (Mainz, Germany) and following the international standard DIN EN ISO 13485 [119]. The Reaxon® is flexible and resistant to collapse, and transparent, which facilitates the insertion of the nerve ends and the application of the anchoring sutures. The electrostatic interaction of the surface of Reaxon® Nerve Guide (positively charged) and the molecules or cells used in nerve repair (negatively charged) promotes the regenerative phenomena [149]. Fornasari et al. conducted a trial study on the mouse model using Reaxon® Nerve Guides, comparing the use of the commercial tube alone with a combination with muscular tissue (for the production of neuregulin 1, a stimulant of activity and survival of Schwann cells) and autografts. Both single tubes and tubes associated with skeletal muscle tissue positively promoted nerve regeneration and return to nerve function [150].

2.1.3 Synthetic nerve conduits

Polymers of synthetic origin have been applied in recent decades as a material for surgical sutures with relative success, and many of them have already been approved for clinical application. These materials present several advantages when used as scaffolds and neural tube guides. First, they can be adapted and produced in a wide variety of mechanical properties and degradation rates. Its well-described characteristics indicate a relatively low risk of immune reactions. Finally, the synthetic polymers can be combined to create new unique mechanical properties. Nevertheless, the biocompatibility of some of these materials may be reduced due to the difficulty of cells to adhere and survive.

Since non-biodegradable tube guides have the disadvantage of requiring a second surgery to remove the implant, research has focused on the development of biodegradable synthetic materials with an acceptable degradation time. The

products resulting from degradation phenomena must not have toxic effects or trigger foreign body reactions. In addition, the biodegradable synthetic materials can be adapted towards the requirements necessary for their purpose, namely, serving as a support for the cellular systems used [151]. Examples of biodegradable synthetic materials are herein described:

2.1.4 Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid), a copolymer resulting from the reaction between the biodegradable poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), is one biomaterial presenting appropriate biocompatibility and biodegradability.

The isolated PGA is a rigid, thermostatic, highly crystalline polyester with high tensile modulus and high melting point but with low solubility in organic solvents. Its degradation leads to the production of glycolic acid, whose detrimental effects on growing cells are limited. Because of its hydrophilic nature, PGA has been used in the past to produce the first fully synthetic absorbable sutures [101]. A crimped PGA tube (Neurotube® Synovis Micro Companies Alliance, Birmingham, AL, USA) was approved as the first synthetic, highly porous, and bioresorbable NGCs approved by FDA in 1999 and was used for the repair of peripheral nerve injuries [152]. More recently, the use of BMSCs and Schwann-like cells (that had differentiated from BMSCs) in combination with Neurotube® in autografted rat facial nerves after neurotmesis was reported. At 6 weeks after surgery and application of the therapeutic combinations, it was found that facial nerve regeneration was improved by the cell therapy associated with PGA tubes [153]. However, PGA may also present some drawbacks. After implantation, ester bonds of the polymer may undergo hydrolysis, leading to degradation and production of derived metabolic products, triggering pH changes in the implantation site after organic absorption [101]. To improve the characteristics, PGA copolymers with PLA were developed, resulting in a more hydrophilic material.

PLA can be produced based on lactic acid obtained from natural products such as wheat, corn, or sugar beet, exhibiting good biocompatibility [154]. The speed of degradation of the scaffolds can be modified by varying the proportion of the different polymers. Lactic acid is more hydrophobic because of an extra methyl group, which not only limits water uptake by about 2% but also decreases hydrolysis rate compared to PGA [101], despite PGA being less soluble in organic solvents than PLA [155]. This biomaterial has been used as NGC in some studies. One study reports the application of PLA NGCs subjected to microabrasion in injured peripheral nerves of rat, promoting the regeneration of the nervous gap 8 weeks after the surgical intervention [156]. In a different study, a PLA nerve conduit obtained through a process of immersion precipitation was used to connect a 20-mm-long lesion in the sciatic nerve of the rabbit. These conduits sustained macropores in their external layer and micropores in their internal layer, allowing a better outflow rate than the inflow rate. This treatment resulted in up to 80% functional recovery after 18 months [157]. In another study, the authors compared the use of nerve conduits comprising PLA nonwoven fabric, silicone tubes filled with type I collagen gel, and autologous nerves in the regeneration of the buccal branch of the facial nerve presenting a 7 mm lesion. At 13 weeks after surgery, myelinization degree and axonal diameter were higher in the PLA tube-treated groups [158]. Still in another study, a microporous micro-patterned PLA obtained by photolithography was tested in the regeneration of a rat sciatic nerve with a 15 mm defect, with observation of good regeneration capacity and functional recovery, particularly when used with neural stem cells on the micro-patterned surfaces [159].

The copolymer of PLA and PGA (PLGA) presents increased hydration and degradation rates than the individual homopolymers [160] and has been described in multiple studies to assess for its ability to promote nerve regeneration [61]. Its most relevant features are its good biodegradability and biocompatibility and possible tuning of degradation time [161]. Its effectiveness is potentiated when combined with growth factors [162] and cell therapies [163, 164] or with the inclusion of a 3D support structure within the conduit [161]. Although these conduits show significantly better results than other biomaterials, they are not easily adaptable for different gap lengths nor for the release of different drugs. There are several drug delivery mechanisms described, such as microspheres, coatings, cross-linked polymers, or lumen filling with different solutions, but there is still little flexibility in the choice of active compounds and their concentrations in different PNI [165].

Over the years, PLGA has been one of the most frequently used biodegradable polymers for biomedical studies. Its degradation leads to the production of glycolic acid, and even if the effect of this product on the cells is reduced, PLGA degradation products are more acidic than other products such as collagen, which may eventually trigger changes in the underlying tissues [154]. Mechanically, its degradation characteristics can be controlled by changes in its molecular weight, copolymer ratio, and crystallinity, allowing for degradation periods varying from months to years, based on the proportion between the two polymers [166].

2.1.5 Poly(D,L-lactide-co-ε-caprolactone)

Poly-ε-caprolactone is an aliphatic, bioabsorbable, and biocompatible polyester commonly used in pharmaceuticals for wound treatment [167]. Its production is achieved by chemical synthesis from crude oil. Since PCL degrades by hydrolyzing its ester linkages under physiological conditions, it has gained prominence among implantable biomaterials. Poly(D,L-lactide-co-ε-caprolactone) (PCL) is a copolymer between the caprolactone and lactic acid monomers, and 80/20 copolymer nerve conduits can be produced by ink-jet systems [168]. Its degradation rate is slower than PLGA (about 16 months). In addition, the PCL degradation products are less acidic than those of PLGA, with less damage to the surrounding tissues. Finally, the PCL is transparent, making it easier to position the nerve stumps. However, its poor flexibility may hamper the microsurgical technique during implantation [164].

One study demonstrated that cells with genetic modifications to release NGF could adhere, survive, and release NGF for extended periods (>8 weeks) when cultured onto 80/20 PLA-PCL scaffolds, while 25/75 and 40/60 PLA-PCL copolymers were deemed unable to sustain cellular adhesion and survival [169]. Other studies demonstrated the in vivo biocompatibility of PCL membranes, tube guides, and nerve cells, facilitating cell adhesion, differentiation, and growth [144, 170].

Neurolac™ (commercially available PCL conduit) application in vivo results in conflicting reports. While some studies have attest the efficacy of PCL to promote both morphological recoveries and functional improvements in neurotmesis and axonotmesis lesions of rat sciatic nerves [170, 171], others report no beneficial effects [172]. Luís et al. compared the effectiveness of PLGA 90/10 and PCL NGCs in helping nerve regeneration along the 10 mm gap of the rat sciatic nerve, further comparing this material with conventional approaches of end-to-end neurorrhaphy and autologous graft. Both types of biomaterials promoted functional improvements and were considered as good options for tubular NGCs, and their degradation characteristics did not seem to have an impact over the level of nerve regeneration. After the 20-week study period, PGLA presented accelerated

biodegradation when compared to PCL [170]. The efficacy of PCL membranes (Vivosorb™) in the promotion of nerve regeneration after neurotmesis was tested in combination with MSCs from the Wharton's jelly (WJMSCs), demonstrating the differentiation of the MSCs into neuroglia-like cells, expressing specific phenotypical markers. In vivo tests performed over 20 weeks resulted in functional recovery and significant morphometric improvements [164].

2.1.6 Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a nondegradable and water-soluble polymer whose potentialities to be used as NGC have recently been explored [173–175]. Currently there is a non-absorbable PVA hydrogel (SaluBridge, SaluTunnel, SaluMedica LCC, Atlanta, GA, USA). Its 3D nanofibrillation structure confers exceptional biocompatibility, water intake capacity, elasticity, mobility and saturability, and high resistance to mechanical deformation [176]. In one study, a tubular PVA conduit was tested for its effects over axonal growth using rat dorsal root ganglia, considering its wall thickness, its level of porosity, and the Schwann cell seeding density. It was identified that lower porosity and higher wall thickness delayed the regeneration of the axons, with the best results observed with 75% porosity, associated with Schwann cell-seeded conduits [177].

More recent studies address the combination of PVA with other materials. Ribeiro et al. studied the effect of PVA loaded with electrically conductive materials (polypyrrole and carbon nanotubes) on axonotmesis injuries. The combination of PVA and carbon nanotubes displayed improved biocompatibility, electrical conductivity, and better histomorphometric results [178]. After neurotmesis injury, the association of MSCs with the PVA, carbon nanotubes promoted better nerve fiber regeneration, suggesting a positive synergistic effect [179].

3. Cellular systems

Historically, neural-derived cellular systems were the first to be proposed for neural tissue regeneration (as the N1E-115) and are briefly addressed herein. Later, MSCs isolated form a variety of niches that have been proposed for the purpose, and some populations are gaining prominence due to technical and ethical minutiae, such as perinatal, dental, and olfactory mucosa-derived MSCs (**Table 2**).

MSC	Classification	Advantages and Mechanism of Action
N1E-115	Neuroblastoma cell line	• Increase the local concentration of neurotrophic factors.
Fetal-derived Mesenchymal Stem Cell	Multipotent stem cells	• Augment the blood perfusion; • Enhance intraneural vascularity; • Differentiate into Schwann-like cells; • Produce and release varied neurotrophic factors; • Collection usually does not require surgical intervention; • Less immunoreactivity.
Dental Pulp Mesenchymal Stem Cells	Multipotent stem cell	• Promote axonal growth and guidance; • Promote and guides myelinization; • Stronger harvesting and proliferation potential; • Great clonogenic potential.
Olfactory Mucosa Mesenchymal Stem Cells	Multipotent stem cell	• Promote neural differentiation; • Produce neurotrophic factors; • Promote maturation of glial cells; • Activate myelination *in vitro*; • Easy collection and isolation.

Table 2.
Summary of characteristics of the cellular systems used by our research group in cell-based therapies for PNI research.

3.1 N1E-115 cells

Neurotrophic factors may stimulate several important components of neural regeneration process, involving the survival and regrowth of sensory and motor nerve fibers. Thus, in vivo Schwann cell differentiation and axon remyelination [180] may vary depending on the method of releasing these factors. The delivery devices must be highly complex to allow controlled release. In this context, N1E-115, a cell line obtained from mouse neuroblastoma C-1300 [181] and able to follow a neurodifferentiation when exposed to dimethylsulfoxide [182], adenosine 3′;5′-cyclic monophosphate [183] or serum removal started to be studied.

These cells were already used in both axonotmesis and neurotmesis lesions because of its capacity to produce and deliver different neurotrophic factors capable to promote axonal regeneration [184]. The advantage in using N1E-115 cells to increase the local concentration of neurotrophic factors is thought to be related to the fact that the concentration of these factors is similar to those observed in endogenous cell production and they are released directly in the proximity of the region under regeneration. The measurement of $[Ca^{2+}]_i$ allowed to establish 48h under differentiation as the appropriated period, presenting N1E-115 cells in this moment characteristics of neurons without entering the process of cell death associated with $[Ca^{2+}]$ modifications [184, 185].

Different studies were performed to infer on the pro-regenerative capacity of the N1E-115 cell line in the peripheral nerve injury, associating these cells with biomaterials such as collagen [104, 111], hybrid chitosan [144], and PLGA 90/10 [186]. Despite all the expected advantages of using biomaterials in association with these cells, the results obtained did not show a special efficacy of N1E-115 cells in promoting nerve regeneration regardless of the type of lesion [144]. Only slight motor improvements were observed with the combination of collagen and N1E-115 in axonotmesis lesions, while no improvements were noted in neurotmesis lesions previously submitted to end-to-end suture. No functional improvements and poor morphometric regeneration was obtained from the use with PLGA [186]. It was hypothesized that the physical presence of N1E-115 cells at nerve scaffolds may have generated a consumption of local blood supplied nutrients and oxygen, arresting the positive effect of local neurotrophic factor release.

3.2 Perinatal mesenchymal stem cells

Perinatal tissues represent the most primitive MSC niches after the embryonic stage and those that have suffered the least genetic alterations because of environmental exposure, aging, or occurrence of pathological changes [187]. Among these tissues, MSCs can be obtained from different sites, namely, umbilical cord blood and stroma (Wharton's jelly), amniotic fluid, and membrane (**Figure 4a**).
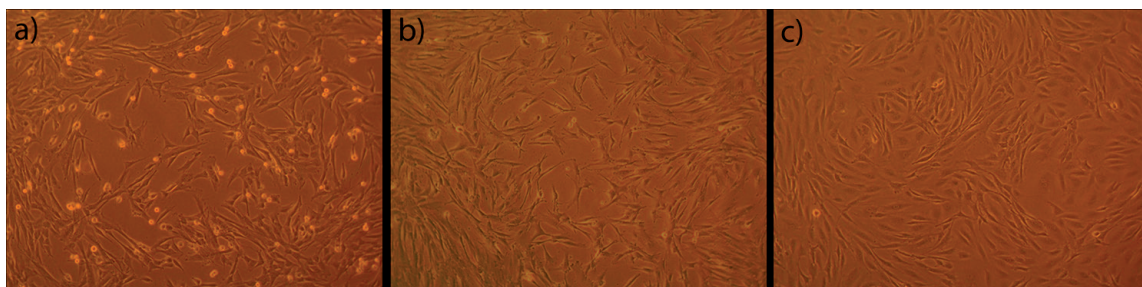
Figure 4.
(a) Equine UC-MSCs in culture (P1). (b) Human dental pulp MSCs in culture (P2). (c) Rat olfactory mucosa MSCs in culture (P4). Magnification: 100×.

Since perinatal tissues are traditionally discarded after birth, MSCs can be isolated through noninvasive procedures. Once isolated, it is easy to establish cultures with these cells and promote their neural differentiation [188, 189]. The list of advantages associated with the use of MSCs with perinatal origin includes the fact that they allow an autologous cell source, are easily processed and cryopreserved, and present low immunogenicity. Besides that, they have a low tumorigenic potential when compared to other types of MSCs [190] and have excellent cell growth capacities. The quality of these cells can vary between patients, depending on the specific characteristics of the tissues of each donor, the transport time, the conditions to which the cells were subjected during the same, and the processing and cryopreservation techniques applied [191].

The umbilical cord stroma (Wharton's jelly) is a singular primitive proteoglycan connective tissue that protects the blood vessels of the umbilical cord and the cells within [192]. The amount of cells that can be isolated from the Wharton's jelly (WJMSCs) is comparatively superior than those that can be isolated from other niches. These cells lack the expression of hematopoietic markers [99, 193], express low levels of histocompatibility complex (MHC) class I, are negative to MHC II, are also easily expanded in culture and plastic adherent, exhibit a normal fibroblastic-like shape [190], and have excellent population doubling times [194]. The in vitro differentiation capacity of these cells is also ample towards several mesodermal cell types such as osteocytes, chondrocytes, adipocytes, skeletal myocytes, cardiomyocytes, hepatocytes, insulin-producing cells, and, of particular importance, neuron-like cells which differentiate when exposed to a neurogenic culture medium for a period of 96 h [190]. WJMSCs have the capacity not only to differentiate into Schwann-like cells but also to produce and release varied neurotrophic factors such as BDNF, NGF, and neurotrophin-3, stimulating axonal growth in vitro [195]. In addition, transformed MSCs still present viability during 4 months after transplantation without any need to institute immune suppression [188].

WJMSCs have already been used to promote the regeneration of different tissues in combination with biomaterials with relative success. Using WJMSCs associated with a PVA membrane to treat chronic cutaneous lesions showed good levels of skin regeneration and reduction in number and extension of ulcers [196]; the use of WJMSCs and their conditioned medium (CM) in association with gelatin matrix scaffolds (commercially available haemostatic sealant, Floseal®) in promoting regeneration of myectomy lesions revealed good functional and histological improvements, although some long-term negative effects were detected and not observed in the treatment with CM. The CM obtained from the culture of WJMSCs can be, therefore, a suitable alternative to the in vivo application of these cells [197].

Regarding the efficacy of WJMSCs in peripheral nerve regeneration after injury, several studies have already been performed. The addition of WJMSCs to NGCs seems to bring advantages related to the production and secretion of neurotrophic and angiogenic factors that improve the local regenerative environment. Animals submitted to neurotmesis present greater functional and sensory improvements when treated with WJMSCS in combination with biodegradable NGCs than when treated with single NGCs [99]. The combined use of WJMSCs with PVA guide tubes loaded with electrically conductive materials (carbon nanotubes and polypyrrole) was able to prevent the occurrence of neurogenic muscular atrophy and reestablish the neuromuscular junction, with the use of MSCs with PVA loaded with carbon nanotubes being effective in inducing a bigger amount of regenerated fibers and thicker myelin sheets. Similarly, the use of PCL and MSCs in neurotmesis lesions immediately submitted to end-to-end sutures also seemed to bring special advantages in terms of motor function recovery, probably because of local secretion of growth factors and cytokines [193].

Still within the use of MSCs from perinatal tissues in peripheral nerve regeneration, treatment of the rat sciatic nerve after neurotmesis with Floseal® as cell vehicle for WJMSCs mediated the Wallerian degeneration stage, improving the subsequent regeneration and the morphology of the nerve fibers. It promoted a lesser extent of fibrosis in the acute phase of the lesion, besides a chronic phase where a greater thickness of the myelin sheets and a larger number of regenerated fibers have been observed. Positive functional and morphological effects in both acute and chronic phases revealed a positive synergistic effect [198].

3.3 Dental pulp mesenchymal stem cells

The dental pulp is the most internal layer of the tooth and is composed of loose connective tissue that includes blood vessels, nerves, and mesenchymal tissue, having an important function in the primary and secondary development of teeth and in resolving pathological processes such as caries [199]. The formation of odontoblasts and the production of dentin in response to severe lesions in the teeth were precisely the first suggestive signs of the presence of MSCs in the dental pulp. Dental pulp mesenchymal stem cells (DPSCs) were isolated for the first time at the beginning of the last decade from a third molar and demonstrated to be able to differentiate into odontoblast-like cells [200]. Over time DPSCs have been isolated from exfoliated deciduous teeth, human permanent and primary teeth, supernumerary teeth [199], and teeth of various nonhuman species [201]. They exhibit all the characteristics of MSCs that make them appropriate options for clinical application, being capable of following multi-lineage differentiation, (including neural differentiation [202]) when under suitable culture conditions; presenting self-renewal capacity [203]; expressing MSC phenotypic markers [204], stemness-related markers, cytoskeleton-related markers [205]; and, as expected, not expressing hematopoietic markers (**Figure 4b**) [203]. Specifically, DPSCs express neural markers [206, 207]; produce neurotrophic factors; stimulate the differentiation, growth, and orientation of growing axons; and differentiate into active and functional neurons [208, 209]. Compared to other types of MSCs, DPSCs have higher clonogenic capacity, high proliferation, and a larger stem/progenitor cell population. DPSC source tissues are, like perinatal tissues, readily collectible without additional harm to the donor or invasive surgical procedures [210], and isolated cells can be used autologously as long as their characteristics are maintained through good isolation and cryopreservation protocols.

DPSCs secrete different trophic factors that stimulate nerve regeneration and showed ability to chemo-attract trigeminal ganglion axons [211], guiding myelin repair and stimulating dorsal root ganglion neurite outgrowth [212, 213]. Besides its efficacy to stimulate regeneration of other tissues [214], some work has been performed to attest the ability of DPSCs to stimulate peripheral nerve regeneration after PNI. The use of silicone tubes filled with DPSCs embedded in collagen gel proved to be effective in promoting regeneration of the rat facial nerve after injury [215]. The same authors further assessed degradable PLGA tubes filled with DPSCs embedded in collagen gel, which were able to stimulate regeneration and functional recovery of the rat sciatic nerve after neurotmesis [216]. The use of collagen devices filled with Schwann-like cells induced from DPSCs also resulted in nerve repair and regeneration in sciatic nerves of rats with 15 mm gap [217]. Martens et al. proved not only the ability of DPSCs to differentiate into Schwann cells with increased glial marker expression and secretion of neurotrophic factors but also the ability of these differentiated cells to promote axonal outgrowth and myelination in 2D or 3D culture conditions in an in vitro model [212]. In addition, the synergistic use of DPSCs and Schwann cells with nerve conduits in solving 15 mm gap lesions in the

rat sciatic nerve was demonstrated to be more effective in restoring nerve conduction velocity than the use of DPSCs and nervous conduits alone [218]. Knowing the importance of oligodendrocyte lineage transcription factor 2 in the oligodendrogenic pathway, Askari et al. were capable to induce the differentiation of DPSCs into oligodendrocytes by transfection of a tetracycline (Tet)-inducible system expressing oligodendrocyte lineage transcription factor 2 gene. These differentiated cells were then used in the treatment of a local demyelinating lesion of the mouse sciatic nerve by lysolecithin, with observation of repair and regeneration of the injured nerve [209]. The comparative use of DPSCs and neuronal cells originating from the differentiation of DPSCs in the treatment of a 5 mm lesion in the rat sciatic nerve proved that both cell types promoted functional and muscle contraction improvements, associated with the identification of specific markers for angiogenesis, even though no specific differences between the two cell types in promoting nerve regeneration were identified [219]. Furthermore, in a model of diabetic polyneuropathy, DPSC transplantation promoted the secretion of several cytokines that were capable of modulating the M1/M2 macrophage proportions and promoting anti-inflammatory effects, besides increasing the velocity of nerve conduction and local nerve blood flow [220].

In summary, DPSCs have the remarkable capacity to not only produce and release neurotrophic factors with protective immune modulative functions at the site of nerve damage but also to differentiate into oligodendrocyte-like and Schwann-like cells.

3.4 Olfactory mucosa mesenchymal stem cells

In the *lamina propria* of mammal olfactory mucosa, a different MSC population can be found: the olfactory mucosa mesenchymal stem cells (OM-MSCs). This lineage has also been called ectomesenchymal stem cells because of their ectodermal origin and the fact that they express neural cell-related genes [221]. The OM-MSCs have been identified and studied in different extents in diverse species such as humans [221], mouse [222], rabbit [223], dog [224], sheep, horse, macaque, and lemur [225], although they were initially found and identified at the rat olfactory mucosa [226]. Some studies were carried out and allowed an initial characterization of these cells, namely, the identification of their MSCs characteristics when in culture (plastic adhesion and capacity to form fibroblastic-like low density colonies) and the expression of classic MSC markers and of those related to differentiations (**Figure 4c**) [221]. In fact, OM-MSCs not only are capable of performing classical tri-differentiation but also have the ability to myogenic and neurogenic differentiation [227]. Additionally, its CM can promote the proliferation of ensheathing cells and oligodendrocyte precursor cells and also activate myelination in vitro [228]. OM-MSCs present features that put them in the list of cells to be used in regenerative medicine, namely, its high versatility, wide distribution in nasal cavity with easy access, few ethical issues, good location for both *antemortem* and *postmortem* collection (particularly in larger donors), neural crest origin, and little tendency for development of chromosomal or tumorigenic alterations [229, 230]. Finally, OM-MSCs maintain self-renewal ability in culture for long periods of time by conserving telomeric activity and inhibiting apoptotic activity, without the age seeming to affect this characteristic [231].

The study of OM-MSCs secretome led to the identification of several molecules with the capacity to promote effects on neural differentiation and production and maturation of glial cells [81]. From a clinical application point of view, besides the studies carried out to test its potential in the control of autoimmune diseases [232, 233] and in the regeneration of myocardial tissue after infarct [234], OM-MSCs

have already shown regenerative efficacy when used as therapy in degenerative diseases of the CNS [235], hippocampal lesions [236, 237], lesions associated with hearing loss [238–240], and in cases of spinal cord trauma [241, 242]. Regarding its effectiveness in promoting peripheral nerve regeneration after PNI, the studies and data obtained are still reduced to allow definitive conclusions. Roche et al. carried out a work in which they studied the efficacy of OM-MSCs delivered in an NGC comprising of a biphasic laminin and collagen-functionalized hyaluronic acid, testing its efficacy on the regeneration of a rat sciatic nerve with a 10 mm gap, with and without NGF supplementation. It was identified that animals treated with OM-MSCs and implantation of NGCs showed clinical and electrophysiological improvements and a nociceptive recovery superior to those identified in the animals that only received NGCs [243].

4. Microsurgical procedures

As in most biomedical works, mouse and rat are the most frequently used models in the studies of peripheral nerve regeneration. Our research group works mainly with the rat model, Sasco Sprague-Dawley breed. The anatomy of the rat is well characterized, presenting many similarities with men's peripheral nerves. Being a small model, the rat still presents nerves with significant dimensions, facilitating the performance of the microsurgical interventions and allowing standardization and comparison of the functional tests performed. In terms of dimensions and density of connective tissue, there are differences in comparison to human nerves, and the biggest disadvantage of using small rodents is their high intrinsic neural regeneration capacity, which can sometimes make it difficult to determine the translational value of the therapeutic methods applied [244, 245]. Regarding the nerve model, most studies explore the sciatic nerve and its terminal branches, particularly because of the dimensions of this nerve [246] and the high number of functional and behavioral tests available, mostly in the rat model [247]. Obviously, the high number of data accessible in the literature allows an effective comparison with the results observed in previous work. The most common types of experimental lesion paradigms include the induction of crush injuries that lead to axonal functional interruption with maintenance of connective sheaths (axonotmesis), disruption of the nerve trunk through a complete transection, or removal of a segment with creation of a gap with specific dimensions (neurotmesis) [248].

In our works, animals are operated under anesthesia and with adequate analgesia. With the animal in lateral decubitus, the trichotomy and asepsis of the area to intervene is performed, and the access to the sciatic nerve is made through a skin incision that extends from the greater trochanter to the distal mid-half of the hind limb and a dissection of the gluteal muscles. Once the sciatic nerve and its main branches are exposed with the aid of soft tissue retractors, the pretended lesion can be induced with a straight microsurgical scissors for neurotmesis lesions or crushing clamps for axonotmesis lesions. The lesion should be induced as distally as possible, preferably directly above the terminal nerve ramification. In neurotmesis lesions, both nerve ends are introduced about 3 mm inside the NGCs with or without associated cells, with the application of 7/0 monofilament nylon epineural sutures to keep nerve tops aligned, secure, and with a gap of desired dimensions. For neurotmesis lesions where there is no intention to maintain a gap, the nerve can be subjected to an end-to-end suture using 7/0 monofilament nylon, with or without posterior application of an NGC. For axonotmesis lesions, the biomaterial can be placed wrapping the crushing site and sutured. The cellular systems can be combined to the biomaterial conduit before (cultured and expanded on the biomaterial surface),

during (infiltrated into the conduit in suspension or in a soft biomaterial vehicle, at the time of implantation), or after reconstruction (through direct injection within the nerve gap). Once this phase is concluded, the gluteal muscles can be sutured, isolated, or simultaneously with the skin, applying a simple-interrupted suture with a non-absorbable material. The contralateral limb is not intervened and used as a control. Animals submitted to surgery should be daily monitored to accompany the healing process and the recovery of nerve function [3].

5. Functional evaluation

In the studies involving sciatic nerve regeneration, the animal is followed postoperatively during 12 weeks for axonotmesis lesions [111] and 20 weeks for neurotmesis lesions [104], assuming that after this period functional and morphologic recovery is complete and can be determined. To determine the level of nerve regeneration, both morphological and functional results are considered in both types of lesions, although the correlation between the two types of data is not always strong [249]. The more classic and modern methods for determining nerve recovery, such as retrograde labeling [250] histomorphometry and histology [251], electrophysiological assessment [252], and in vivo imaging [253], rarely succeed in adequately revealing the reestablishment of motor and sensory functions, being more effective in the study of the regenerative process from the physiologic/structural point of view than in the assessment of effective functional recovery [254]. Thus, PNI research studies need to combine both functional and morphological assessment. Our works generally involves conducting a behavioral analysis, combined with histology, histomorphometry, and kinematic analysis (**Figure 5**).

5.1 Behavioral and functional analysis

Regarding motor function of the sciatic nerve, the test used to determine its recovery is the evaluation of SFI through the use of a walking track. First described in 1982 [255], the SFI is a quantitative and noninvasive method that allows to determine the recovery of the hind limb using methods of observation and recording of the rat's footprints, considering for this the spatial relation between the toes, the foot, and the hind limb as a whole [256]. Although it is a very popular test among PNI researchers, its validity is still questioned [257]. The major limitation
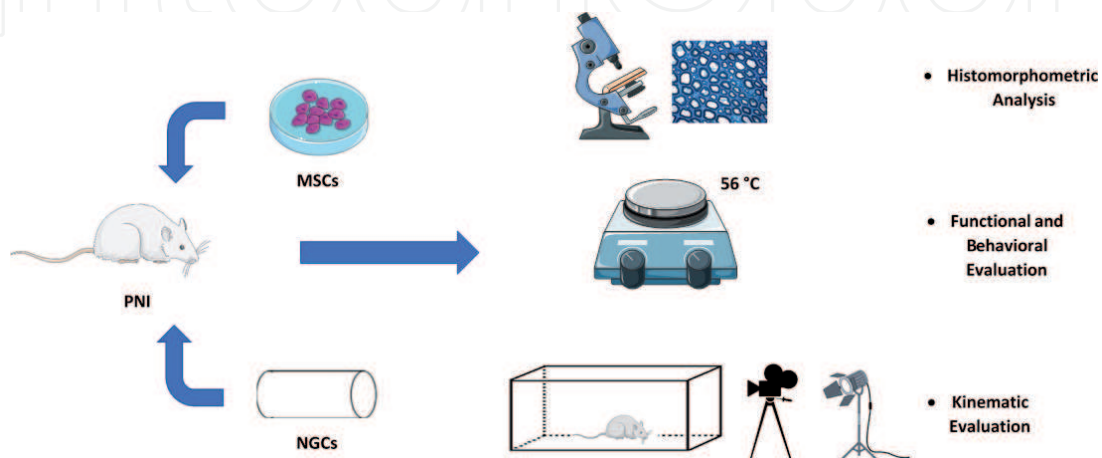
Figure 5.
Schematic representation of evaluation components used in works of our research group.

of the method is that animals frequently develop contractile flexions and autotomy, which consequently leads to defective and blurred paw records because of changes in limb placement or tail dragging during the footprint record, making it difficult to analyze [258]. To perform the test, the animals are confined within a 42-cm-long and 8.2-cm-wide walkway that ends in a dark shelter without exit. A white paper is placed on the floor of the walking corridor for registration. The hind paws of the rats are gently pressed onto the finger paint-soaked sponge to be impregnated with ink, and the animals are then placed at the beginning of the walkway to advance along the corridor and leave their hind paw records over the paper. Animals are always trained to walk in the corridor prior to surgery to stablish an individual baseline. Walking tracks are recorded preoperatively (week 0), after surgery at weeks 1 and 2, and from there every 2 weeks until week 12 or 20. In each record it is possible to make several measurements: print length (PL), distance from the heel to the third toe; toe spread (TS), distance from the first to the fifth toe; and intermediate toe spread (ITS), distance from the second to the fourth toe. Associated with the SFI, SSI is also determined, in which only the TS and ITS parameters are considered. SSI and SFI measurements are made on both the control and injured limbs. The average of three measurements are used in the following formulas: Toe spread factor (TSF) = (ETS − NTS)/NTS; Intermediate toe spread factor (ITSF) = (EITS − NITS)/NITS; Print length factor (PLF) = (EPL − NPL)/NPL, with E and N representing the injured and non-injured limbs, respectively. Finally, the SFI is calculated using the formula of Bian et al. [259]: SFI = −38.3 (EPL − NPL)/NPL + 109.5 (ETS − NTS)/NTS + 13.3 (EIT − NIT)/NIT − 8.8 = (−38.3 × PLF) + (109.5 × TSF) + (13.3 × ITSF) − 8.8. Alternatively, or complementarily, SSI is a fast index that is calculated without considering the PL value, using the equation: SSI = [(108.44 × TSF) + (31.85 × ITSF)] − 5.49. For both cases, a value of 0 is normal, and the closer the value is to −100, the worse the functional recovery. In situations where footprints cannot be measured, the value of −100 is automatically assigned. The footprints of each animal must be observed and analyzed by a single operator.

The method to test the motor performance is the EPT test [260], which consists in determining the force, measured in grams, that the animal is capable to exert with the injured and healthy limbs over a digital scale. This is an important test because for its correct accomplishment the animal needs to activate the muscles of the plantar flexor group (gastrocnemius and soleus), and the obtained values are correlated with those observed in the SFI and SSI [257]. To perform this test, the animal's body is wrapped in a surgical towel, leaving the hind limbs exposed. The animal must be supported by the thorax as it is lowered towards the digital balance. As hind limbs approach the balance, the EPT is elicited by the anticipation of the contact of the distal metatarsus with the balance, and the hind limbs are extended. The force, in grams, exerted over the balance is then registered. The method should be performed on both the affected and the healthy limb, being repeated three times to consider the average result. To determine the functional deficit percentage, normal limb (NEPT) and injured limb (EEPT) EPT values are then included in the following equation [261]: Percentage motor deficit = [(NEPT − EEPT) /NEPT] × 100. Animals are tested before surgery (week 0), week 1 and 2 after surgery, and from there every 2 weeks to week 12 or 20. EPT values are originally determined in grams of weight applied by each limb but are subsequently expressed as percentage deficit of the injured limb relatively to the weight applied by the healthy limb. The main limitations of the test relate to the operator's experience and comfort in handling animals. The operator also needs to have enough experience to recognize when the animal is applying as much force as possible with the limb to be tested. Since the operator is comfortable performing the test, it is highly reproducible [261].

Regarding the sensorial recovery of the sciatic nerve, the most used test is the WRL, also used in works of our research group. The WRL test, with the aim of establishing the maintenance or recovery of the nociceptive function, can be performed using a mechanical stimulation and electrical stimulation, by puncturing with a needle or, more commonly, with a heating plate [262]. Using a hot plate is not only the most common but also the most practical method. The nociceptive stimulation is applied on the hindpaw's plantar surface, determining the withdrawal reflex, that is, the time, in seconds, that the animal takes to retract the paw. Animals with sensory integrity retract the limbs more quickly from the nociceptive stimulus source [263]. To perform the test, the animal is covered with a surgical towel, and the limb to be tested is placed over the hotplate at 56°C, the time it takes to retract the limb being then recorded. In a healthy animal, the limb is retracted in around 4.3 s or less [264]. Limbs are tested three times, with a 2-min interval between each test to avoid sensitizations, and the result of three measurements is considered, on average, to get the final result. If the animal does not retract the limb within 12 s, it is removed from the heat stimulus to prevent tissue damage. The animals are tested before surgery (week 0), at week 1 and 2 after surgery, and from there every 2 weeks until week 12 or 20.

5.2 Kinematic analysis

The evaluation of locomotion quality is of utmost importance since this function integrates the sensory and motor systems and their constituents, including the nervous fibers of afferent and efferent nature, the sensory nerve terminations, the skeletal muscles, and the respective central integration centers. With the convenience of advanced image record devices, it is now possible to use digital technologies to more accurately evaluate gait analysis [265]. The kinematic evaluation is the set of analyses directed to the articular movements without considering the force that is being applied. Considering that branches of the sciatic nerve are responsible for the innervation of dorsiflexor and plantarflexor muscles, the set of kinematic evaluations used in this nerve, with video capture and later observation, include the determination of ankle kinematic, the measurement of gait stance duration, and evaluation of toe out angle during the gait [257]. The main disadvantage of kinematic evaluation is its technical complexity and the need for specific digital material.

In the studies conducted by our group (**Figure 6**), the kinematic evaluation of the ankle is performed considering the sagittal plane during the stance phase of walking after the induction of different injuries [104, 111]. Animals are encouraged to walk voluntarily throughout a corridor with two dark shelters at both ends to serve as a refuge, thus allowing the two-dimensional ankle motion analysis. The side walls of the corridors are transparent, and a high-speed video camera is positioned in an orthogonal position relatively to the corridor to record the ankle motion during the walk. Sagittal records are also considered, using a rate of 100 frames per second. The recorded images are scanned in a semiautomatic process resorting to marks placed at reference points over the rat hind limb and paw. By this procedure it is possible to get the trajectories of the leg and hindfoot segments, and the ankle joint angle is derived by using appropriate computation systems. The parameters for ankle kinematics proposed by Varejão et al. [266] are then applied to determine the sciatic functional recovery after injury and repair, so that therapeutic efficacies can be compared.

It is important, however, to realize that ankle kinematics should only be regarded as an indirect sign of muscle function. Animal locomotion requires the use of fine coordination of limbs, and quadruped animals can develop mobility strategies to compensate deficits in hind limbs. Through the plastic activation of integrative structures, the animal can develop patterns of adaptive movement that are observed
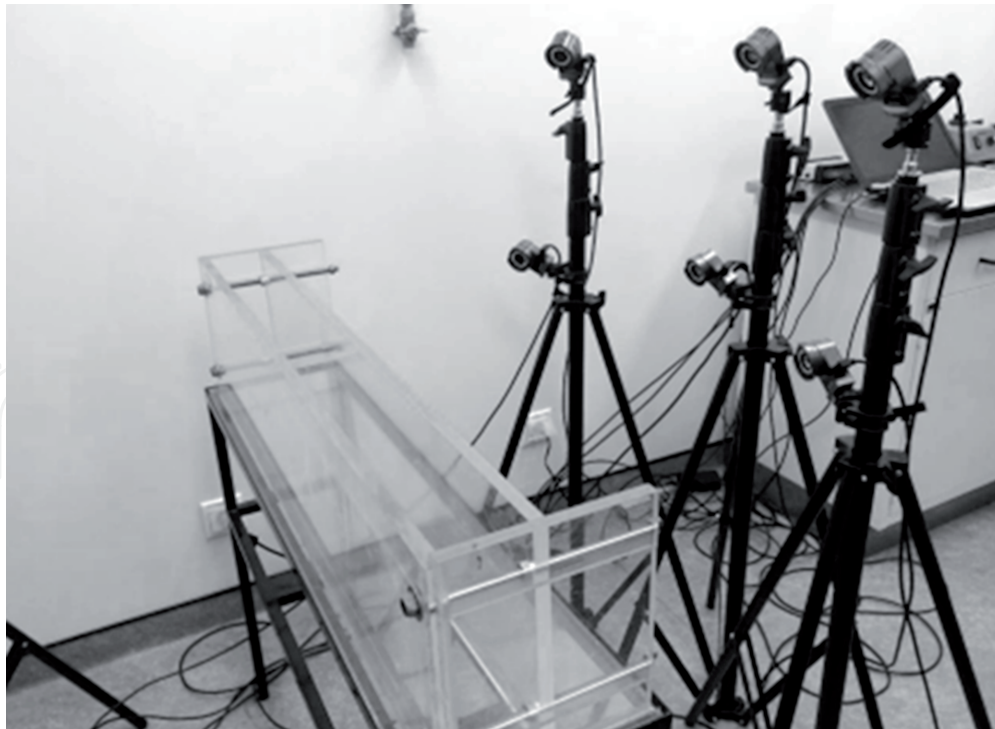
Figure 6.
Corridor and data capture chamber setup for kinematic evaluation in works of our research group.

even in the presence of severe denervation. In addition, a direct relationship between the results of simpler motor and sensory function tests and those got in the evaluation of the complex walking action is rarely observed [266, 267]. To achieve a precise assessment of functional recovery, walking analysis after PNI should evolve both the ankle kinematics analysis to a detailed description of the biomechanical and the mobility function of the hind limb, including a complete assessment of hip, knee, and ankle joints.

6. Morphological analysis

The morphological and histological evaluation of the injured nerve after the experimental period is a commonly used method to identify the size, organization, and number of regenerated nerve fibers and the thickness of the myelin sheath formed after PNI. Although morphological and histological evaluation was only descriptive in its earlier applications, it is now possible to perform morphometric and quantitative analysis of the histological sections to be studied, ideally in combination with other alternative methods of functional, electrophysiological, and molecular evaluation [251]. Quantitative analysis is important to identify both intact and regenerated axons, inflammation, and fibrotic reactions inside the nerve or in the form of perineural adhesions, besides the development of neuromas. The histomorphometric assessment also allows to identify the amount, type, and diameter of the cells that occupy a certain space within the nerve and the proportion of regenerated and healthy tissue [268]. When the efficacy of biomaterials is assessed, histological evaluation is essential to determine the level of material degradation, the development of granulomas and adherences, and the establishment of foreign body reactions [39].

The toluidine blue staining of semithin sections is the method more commonly applied for the histological characterization of the nerve after PNI. This technique allows the observation of the myelinated axons and to delimitate the myelin

sheaths. Likewise, this method is adequate to perform a morphometric examination that leads to the determination of the density and number of nerve fibers, the cross-section dimensions, the perimeter of fibers and axons, the diameter of the fibers and axons, the different proportions between the axon diameters, fibers and myelin sheaths, and also the thickness of myelin sheaths [269]. In addition, this method also enables the evaluation of the ultrastructural changes caused by the regenerative phenomena in axons and in the myelin through transmission electron microscopy [270].

Regardless of the protocol considered, the histological and morphological evaluation of the nerve requires consolidated experience from the operator. It is necessary that the operator has a thorough knowledge about the anatomy of the nerve, about the histology of its segments, and about the differences between the same nerve sites in different animals. These knowledges are essential during the determination of the dimensions and number of myelinated fibers. The use of randomized protocols, biased measurements, and biased counting methodologies allows to prevent the occurrence of bias in the histological and morphometric evaluations. The morphological methods used to test axonal regeneration do not always allow to directly correlate the functional recovery and the level of axonal regeneration, and appropriate axonal regeneration and low functional outcomes are common occurrences. Moreover, because of the occurrence of protruding, separation, divergence, kinking, or straddling observed between the two portions of axons, histological evaluation can hinder the assessment of the nerve reparation. Finally, even with the high-resolution optical microscopy, myelinated fibers with a diameter infersior to 2 µm are hard to detect, potentially misjudging the counting [271].

7. Conclusions

PNI continue to bear enormous impact on the patient's quality of life, leading to significant functional deficits, disabilities, and substantial social and professional constraints. Significant advances in neural reparation and translational neurophysiology have been achieved through the refinement of microsurgery techniques, the comprehension of anatomy and topography of the nerve, and the understanding of pathophysiologic and molecular mechanisms related to PNI. Nerve reparation by epineural neurorrhaphy is still the preferred yet invasive approach in situations where tension-free alignment in a well-vascularized environment can be guaranteed. For larger gaps between the two nerve segments, this technique is not always adequate, and nerve grafting presents as the treatment of choice. In severe avulsion injuries, such as in the brachial plexus, nerve transfer techniques are also an option. The current most promising research lines in nerve regeneration are based on attempted strategies to accelerate reparation using sophisticated nerve conduits in combination with cell-based therapies. Several types of biomaterials with different physical presentations have been explored, demonstrating attractive pro-regenerative properties. This therapeutic potential is further boosted through its combination with cells, CM, and growth factors, indicating that combinatory therapies are the most promising strategy in regenerative medicine and PNI.

This chapter summarizes the principles of peripheral nerve injury and the observations gathered by our research group in this field over the years. This gathered knowledge results from the exploitation of diverse hypothesized therapeutic combination based on the use of biomaterials and cellular systems, achieving promising results. Nonetheless, there is still a long road ahead in this research area towards the achievement of optimal PNI recovery, and conclusions presented in

currently available literature provide basis for further studies necessary for the consolidation of the proposed therapies.

Acknowledgements

Conflict of interest

The authors of this paper do not declare any conflict of interest regarding the content of the document.

Acronyms and abbreviations

AFMSCs	amniotic fluid mesenchymal stem cells
ALP	alkaline phosphatase
BDNF	brain-derived neurotrophic factor
BMSCs	bone marrow MSCs
CM	conditioned medium
DPSCs	dental pulp mesenchymal stem cells
EPT	extensor postural thrust
GDNF	glial cell-derived neurotrophic factor
GPTMS	glycidoxypropyltrimethoxysilane
ITS	intermediate toe spread
ITSF	intermediate toe spread factor
MHC	histocompatibility complex
MSC's	mesenchymal stem cells
NGCs	nerve guidance conduits
NGF	nerve growth factor
OM-MSCs	olfactory mucosa mesenchymal stem cell
PCL	poly(D,L-lactide-co-ε-caprolactone)
PGA	poly(glycolic acid)
PGA	polyglycolic acid
PL	print length
PLA	poly(lactic acid)
PLF	print length factor

PLGA	poly(lactic-co-glycolic acid)
PNI	peripheral nerve injury
pRb	retinoblastoma gene product
PVA	polyvinyl alcohol
SFI	sciatic functional index
SSI	static sciatic index
TS	toe spread
TSF	toe spread factor
WJMSCs	Wharton's jelly mesenchymal stem cells
WRL	withdrawal reflex latency

Author details

Rui Damásio Alvites[1,2], Mariana Vieira Branquinho[1,2], Ana Rita Caseiro[1,2,3],
Sílvia Santos Pedrosa[1,2], Ana Lúcia Luís[1,2], Stefano Geuna[4],
Artur Severo Proença Varejão[5,6] and Ana Colette Maurício[1,2]*

1 Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Porto, Portugal

2 Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Porto, Portugal

3 Escola Universitária Vasco da Gama (EUVG), Coimbra, Portugal

4 Department of Clinical and Biological Sciences, Cavalieri Ottolenghi Neuroscience Institute, University of Turin, Turin, Italy

5 Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

6 CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

*Address all correspondence to: ana.colette@hotmail.com

IntechOpen

References

[1] Fathi SS, Zaminy A. Stem cell therapy for nerve injury. World Journal of Stem Cells. 2017;**9**(9):144

[2] Sullivan R, Dailey T, Duncan K, Abel N, Borlongan CV. Peripheral nerve injury: Stem cell therapy and peripheral nerve transfer. International Journal of Molecular Sciences. 2016;**17**(12):2101

[3] Taylor CA, Braza D, Rice JB, Dillingham T. The incidence of peripheral nerve injury in extremity trauma. American Journal of Physical Medicine & Rehabilitation. 2008;**87**(5):381-385

[4] Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. Journal of Trauma and Acute Care Surgery. 1998;**45**(1):116-122

[5] Zhang X, Chen WW, Huang WJ. Chemotherapy-induced peripheral neuropathy. Biomedical Reports. 2017;**6**(3):267-271

[6] Pradat P-F, Delanian S. Late radiation injury to peripheral nerves. In: Handbook of Clinical Neurology. Vol. 115. Netherlands: Elsevier; 2013. pp. 743-758. DOI: 10.1016/B978-0-444-52902-2.00043-6

[7] Schreiber AK, Nones CF, Reis RC, Chichorro JG, Cunha JM. Diabetic neuropathic pain: Physiopathology and treatment. World Journal of Diabetes. 2015;**6**(3):432

[8] Antoine J-C, Camdessanché J-P. Peripheral nervous system involvement in patients with cancer. The Lancet Neurology. 2007;**6**(1):75-86

[9] Rasulić L, Savić A, Vitošević F, Samardžić M, Živković B, Mićović M, et al. Iatrogenic peripheral nerve injuries—Surgical treatment and outcome: 10 years' experience. World Neurosurgery. 2017;**103**:841-51.e6

[10] Antoniadis G. The peripheral nerve: Neuroanatomical principles before and after injury. In: Modern Concepts of Peripheral Nerve Repair. Unites States: Springer International Publishing; 2017. pp. 1-10. DOI: 10.1007/978-3-319-52319-4_1

[11] Catala M, Kubis N. Gross anatomy and development of the peripheral nervous system. In: Handbook of Clinical Neurology. Vol. 115. Netherlands: Elsevier; 2013. pp. 29-41. DOI: 10.1016/B978-0-444-52902-2.00003-5

[12] Pereira JA, Lebrun-Julien F, Suter U. Molecular mechanisms regulating myelination in the peripheral nervous system. Trends in Neurosciences. 2012;**35**(2):123-134

[13] Mizisin AP, Weerasuriya A. Homeostatic regulation of the endoneurial microenvironment during development, aging and in response to trauma, disease and toxic insult. Acta Neuropathologica. 2011;**121**(3):291-312

[14] Piña-Oviedo S, Ortiz-Hidalgo C. The normal and neoplastic perineurium: A review. Advances in Anatomic Pathology. 2008;**15**(3):147-164

[15] Lundborg G, Rydevik B. Effects of stretching the tibial nerve of the rabbit. A preliminary study of the intraneural circulation and the barrier function of the perineurium. Journal of Bone and Joint Surgery. 1973;**55**(2):390-401

[16] Peltonen S, Alanne M, Peltonen J. Barriers of the peripheral nerve. Tissue Barriers. 2013;**1**(3):e24956

[17] Olsson Y. Studies on vascular permeability in peripheral nerves.

I. Distribution of circulating fluorescent serum albumin in normal, crushed and sectioned rat sciatic nerve. Acta Neuropathologica. 1966;**7**(1):1-15

[18] Burnett MG, Zager EL. Pathophysiology of peripheral nerve injury: A brief review. Neurosurgical Focus. 2004;**16**(5):1-7

[19] Hainline BW. Peripheral nerve injury in sports. CONTINUUM: Lifelong Learning in Neurology. 2014;**20**(6, Sports Neurology):1605-1628

[20] Lim TK, Shi XQ, Johnson JM, Rone MB, Antel JP, David S, et al. Peripheral nerve injury induces persistent vascular dysfunction and endoneurial hypoxia, contributing to the genesis of neuropathic pain. Journal of Neuroscience. 2015;**35**(8):3346-3359

[21] Navarro X. Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: A critical overview. European Journal of Neuroscience. 2016;**43**(3):271-286

[22] Seddon H. Three types of nerve injury. Brain. 1943;**66**(4):237-288

[23] Sunderland S. A classification of peripheral nerve injuries producing loss of function. Brain. 1951;**74**(4):491-516

[24] Mackinnon S, Dellon A. Diagnosis of nerve injury. In: Surgery of the Peripheral Nerve. New York: Thieme; 1988. pp. 74-79

[25] Lutz AB, Barres BA. Contrasting the glial response to axon injury in the central and peripheral nervous systems. Developmental Cell. 2014;**28**(1):7-17

[26] Faroni A, Mobasseri SA, Kingham PJ, Reid AJ. Peripheral nerve regeneration: Experimental strategies and future perspectives. Advanced Drug Delivery Reviews. 2015;**82**:160-167

[27] Scheib J, Höke A. Advances in peripheral nerve regeneration. Nature Reviews Neurology. 2013;**9**(12):668

[28] Jonsson S, Wiberg R, McGrath AM, Novikov LN, Wiberg M, Novikova LN, et al. Effect of delayed peripheral nerve repair on nerve regeneration, Schwann cell function and target muscle recovery. PloS One. 2013;**8**(2):e56484

[29] Walsh S, Midha R. Practical considerations concerning the use of stem cells for peripheral nerve repair. Neurosurgical Focus. 2009;**26**(2):E2

[30] Alvites RD, Santos ARC, Varejão ASP, de Castro Osório ACP. Olfactory mucosa mesenchymal stem cells and biomaterials: A new combination to regenerative therapies after peripheral nerve injury. Mesenchymal Stem Cells-Isolation, Characterization and Applications: Rijeka InTech; 2017

[31] Carroll S, Worley S. Wallerian degeneration. In: Reference Module in Neuroscience and Biobehavioral Psychology. Netherlands: Elsevier; 2017. pp. 1-8. DOI: 10.1016/B978-0-12-809324-5.02077-0

[32] Hall S. The response to injury in the peripheral nervous system. The Journal of Bone and Joint Surgery British Volume. 2005;**87**(10):1309-1319

[33] Waller AV. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Philosophical Transactions of the Royal Society of London. 1850;**140**:423-429

[34] Dubový P, Klusáková I Hradilová SI. Inflammatory profiling of Schwann cells in contact with growing axons distal to nerve injury. BioMed Research International. 2014;(7):1-7. DOI: 10.1155/2014/691041

[35] Perrin FE, Lacroix S, Avilés-Trigueros M, David S. Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1α and interleukin-1β in Wallerian degeneration. Brain. 2005;**128**(4):854-866

[36] Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, et al. Histology of the peripheral nerve and changes occurring during nerve regeneration. International Review of Neurobiology. 2009;**87**:27-46

[37] Menorca RM, Fussell TS, Elfar JC. Peripheral nerve trauma: Mechanisms of injury and recovery. Hand Clinics. 2013;**29**(3):317

[38] Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, et al. Repairing injured peripheral nerves: Bridging the gap. Progress in Neurobiology. 2010;**92**(3):245-276

[39] Alvites R, Rita Caseiro A, Santos Pedrosa S, Vieira Branquinho M, Ronchi G, Geuna S, et al. Peripheral nerve injury and axonotmesis: State of the art and recent advances. Cogent Medicine. 2018;**5**(1):1466404

[40] Houschyar K, Momeni A, Pyles M, Cha J, Maan Z, Duscher D, et al. The role of current techniques and concepts in peripheral nerve repair. Plastic Surgery International. 2016;**2016**:1-8. DOI: 10.1155/2016/4175293

[41] Au NPB, Kumar G, Asthana P, Tin C, Mak YL, Chan LL, et al. Ciguatoxin reduces regenerative capacity of axotomized peripheral neurons and delays functional recovery in pre-exposed mice after peripheral nerve injury. Scientific Reports. 2016;**6**:26809

[42] Trojaborg W. Rate of recovery in motor and sensory fibres of the radial nerve: Clinical and electrophysiological aspects. Journal of Neurology, Neurosurgery & Psychiatry. 1970;**33**(5):625-638

[43] Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: Gaining perspective on inflammatory events after peripheral nerve injury. Journal of Neuroinflammation. 2011;**8**(1):110

[44] Höke A. Mechanisms of disease: What factors limit the success of peripheral nerve regeneration in humans? Nature Reviews Neurology. 2006;**2**(8):448

[45] Saied A, Shekaari MA, Sadeghifar A, Karbalaeikhani A. Introduction of a new suture method in repair of peripheral nerves injured with a sharp mechanism. Archives of Bone and Joint Surgery. 2015;**3**(4):254

[46] Dahlin L. Techniques of peripheral nerve repair. Scandinavian Journal of Surgery. 2008;**97**(4):310-316

[47] Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. BioMed Research International. 2014;**2014**:1-13. DOI: 10.1155/2014/698256

[48] Jerome JTJ. Anterior deltopectoral approach for axillary nerve neurotisation. Journal of Orthopaedic Surgery. 2012;**20**(1):66-70

[49] Rohde RS, Wolfe SW. Nerve transfers for adult traumatic brachial plexus palsy (brachial plexus nerve transfer). HSS Journal. 2007;**3**(1):77-82

[50] Simon NG, Spinner RJ, Kline DG, Kliot M. Advances in the neurological and neurosurgical management of peripheral nerve trauma. Journal of Neurology, Neurosurgery, and Psychiatry. 2016;**87**(2):198-208

[51] Matsuyama T, Mackay M, Midha R. Peripheral nerve repair and grafting techniques: A review.

Neurologia Medico-chirurgica. 2000;**40**(4):187-199

[52] Lundborg G. Bridging nerve defects-the role of tissue interpositioning. In: Severe Traumatic Defects of the Upper Limb. USA: CRC Press; 2004. pp. 151-165

[53] Millesi H. Bridging defects: Autologous nerve grafts. In: Acta Neurochir Suppl. Vol. 100. Austria: Springer-Verlag; 2007. pp. 37-38. DOI: 10.1007/978-3-211-72958-8_8

[54] Grand AG, Myckatyn TM, Mackinnon SE, Hunter DA. Axonal regeneration after cold preservation of nerve allografts and immunosuppression with tacrolimus in mice. Journal of Neurosurgery. 2002;**96**(5):924-932

[55] Safa B, Buncke G. Autograft substitutes: Conduits and processed nerve allografts. Hand Clinics. 2016;**32**(2):127-140

[56] Yi J-S, Lee H-J, Lee H-J, Lee I-W, Yang J-H. Rat peripheral nerve regeneration using nerve guidance channel by porcine small intestinal submucosa. Journal of Korean Neurosurgical Society. 2013;**53**(2):65

[57] Madduri S, Feldman K, Tervoort T, Papaloïzos M, Gander B. Collagen nerve conduits releasing the neurotrophic factors GDNF and NGF. Journal of Controlled Release. 2010;**143**(2):168-174

[58] Kehoe S, Zhang X, Boyd D. FDA approved guidance conduits and wraps for peripheral nerve injury: A review of materials and efficacy. Injury. 2012;**43**(5):553-572

[59] Babu P, Behl A, Chakravarty B, Bhandari P, Bhatti T, Maurya S. Entubulation techniques in peripheral nerve repair. Indian Journal of Neurotrauma. 2008;**5**(01):15-20

[60] Muheremu A, Ao Q. Past, present, and future of nerve conduits in the treatment of peripheral nerve injury. BioMed Research International. 2015;**2015**:237507

[61] Daly W, Yao L, Zeugolis D, Windebank A, Pandit A. A biomaterials approach to peripheral nerve regeneration: Bridging the peripheral nerve gap and enhancing functional recovery. Journal of the Royal Society Interface. 2012;**9**(67):202-221

[62] Pettersson J, McGrath A, Kalbermatten DF, Novikova LN, Wiberg M, Kingham PJ, et al. Muscle recovery after repair of short and long peripheral nerve gaps using fibrin conduits. Neuroscience Letters. 2011;**500**(1):41-46

[63] Simões MJ, Amado S, Gärtner A, Armada-da-Silva PA, Raimondo S, Vieira M, et al. Use of chitosan scaffolds for repairing rat sciatic nerve defects. Italian Journal of Anatomy and Embryology. 2010;**115**(3):190-210

[64] Scatena M, Eaton KV, Jackson MF, Lund SA, Giachelli CM. Macrophages: The bad, the ugly, and the good in the inflammatory response to biomaterials. In: The Immune Response to Implanted Materials and Devices. Switzerland: Springer International Publishing; 2017. pp. 37-62. DOI: 10.1007/978-3-319-45433-7_3

[65] Nectow AR, Marra KG, Kaplan DL. Biomaterials for the development of peripheral nerve guidance conduits. Tissue Engineering Part B: Reviews. 2011;**18**(1):40-50

[66] Basu B. Corrosion and degradation of implantable biomaterials. In: Biomaterials for Musculoskeletal Regeneration. Singapore: Springer Nature; 2017. pp. 253-289. DOI: 10.1007/978-981-10-3059-8_8

[67] Belanger K, Dinis TM, Taourirt S, Vidal G, Kaplan DL, Egles C. Recent strategies in tissue engineering for guided peripheral nerve regeneration. Macromolecular Bioscience. 2016;**16**(4):472-481

[68] Meek MF, Coert JH. US Food and Drug Administration/Conformit Europe-approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. Annals of Plastic Surgery. 2008;**60**(1):110-116

[69] Peng S-W, Li C-W, Chiu M, Wang G-J. Nerve guidance conduit with a hybrid structure of a PLGA microfibrous bundle wrapped in a micro/nanostructured membrane. International Journal of Nanomedicine. 2017;**12**:421

[70] Kaur G. Biomaterials influencing human lives. In: Bioactive Glasses. Switzerland: Springer International Publishing; 2017. pp. 1-20. DOI: 10.1007/978-3-319-45716-1_1

[71] Kokai LE, Lin Y-C, Oyster NM, Marra KG. Diffusion of soluble factors through degradable polymer nerve guides: Controlling manufacturing parameters. Acta Biomaterialia. 2009;**5**(7):2540-2550

[72] Xu J, Varitimidis SE, Fisher KJ, Tomaino MM, Sotereanos DG. The effect of wrapping scarred nerves with autogenous vein graft to treat recurrent chronic nerve compression. The Journal of Hand Surgery. 2000;**25**(1):93-103

[73] Suematsu N. Tubulation for peripheral nerve gap: Its history and possibility. Microsurgery. 1989;**10**(1):71-74

[74] Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Molecular Neurobiology. 2003;**27**(3):277-323

[75] Gaudin R, Knipfer C, Henningsen A, Smeets R, Heiland M, Hadlock T. Approaches to peripheral nerve repair: Generations of biomaterial conduits yielding to replacing autologous nerve grafts in craniomaxillofacial surgery. BioMed Research International. 2016;**2016**:1-18. DOI: 10.1155/2016/3856262

[76] Sariguney Y, Yavuzer R, Elmas C, Yenicesu I, Bolay H, Atabay K. Effect of platelet-rich plasma on peripheral nerve regeneration. Journal of Reconstructive Microsurgery. 2008;**24**(03):159-167

[77] Orbay H, Uysal AC, Hyakusoku H, Mizuno H. Differentiated and undifferentiated adipose-derived stem cells improve function in rats with peripheral nerve gaps. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2012;**65**(5):657-664

[78] Hood B, Levene HB, Levi AD. Transplantation of autologous Schwann cells for the repair of segmental peripheral nerve defects. Neurosurgical Focus. 2009;**26**(2):E4

[79] Gao F, Chiu S, Motan D, Zhang Z, Chen L, Ji H, et al. Mesenchymal stem cells and immunomodulation: Current status and future prospects. Cell Death & Disease. 2017;**7**(1):e2062

[80] Al-Zer H, Kalbouneh H. Dental pulp stem cells-derived schwann cells for peripheral nerve injury regeneration. Neural Regeneration Research. 2015;**10**(12):1945

[81] Ge L, Jiang M, Duan D, Wang Z, Qi L, Teng X, et al. Secretome of olfactory mucosa mesenchymal stem cell, a multiple potential stem cell. Stem Cells International. 2016;**2016**:1-16. DOI: 10.1155/2016/1243659

[82] Talwadekar MD, Kale VP, Limaye LS. Placenta-derived mesenchymal stem cells possess better

immunoregulatory properties compared to their cord-derived counterparts—A paired sample study. Scientific Reports. 2015;**5**:15784

[83] Watson N, Divers R, Kedar R, Mehindru A, Mehindru A, Borlongan MC, et al. Discarded Wharton jelly of the human umbilical cord: A viable source for mesenchymal stromal cells. Cytotherapy. 2015;**17**(1):18-24

[84] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[85] Bhangra KS, Busuttil F, Phillips JB, Rahim AA. Using stem cells to grow artificial tissue for peripheral nerve repair. Stem Cells International. 2016;**2016**:1-18. DOI: 10.1155/2016/7502178

[86] Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): A systematic review and meta-analysis of clinical trials. PLoS One. 2012;**7**(10):e47559

[87] Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. Nature Biotechnology. 2014;**32**(3):252

[88] Grinnemo KH, Mansson A, Dellgren G, Klingberg D, Wardell E, Drvota V, et al. Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infarcted rat myocardium. Journal of Thoracic and Cardiovascular Surgery. 2004;**127**(5):1293-1300

[89] Xia Z, Ye H, Choong C, Ferguson DJ, Platt N, Cui Z, et al.

Macrophagic response to human mesenchymal stem cell and poly(epsilon-caprolactone) implantation in nonobese diabetic/severe combined immuno-deficient mice. Journal of Biomedical Materials Research Part A. 2004;**71**(3):538-548

[90] Moll G, Rasmusson-Duprez I, von Bahr L, Connolly-Andersen AM, Elgue G, Funke L, et al. Are therapeutic human mesenchymal stromal cells compatible with human blood? Stem Cells. 2012;**30**(7):1565-1574

[91] Pig J, Ishihara A, Wellman ML, Russell DS, Bertone A. Inflammatory effects of autologous, genetically modified autologous, allogeneic, and xenogeneic mesenchymal stem cells after intra-articular injection in horses. Veterinary and Comparative Orthopaedics and Traumatology. 2013;**26**(06):453-460

[92] Joswig AJ, Mitchell A, Cummings KJ, Levine GJ, Gregory CA, Smith R 3rd, et al. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. Stem Cell Research & Therapy. 2017;**8**(1):42

[93] Von Bahr L, Batsis I, Moll G, Hägg M, Szakos A, Sundberg B, et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells. 2012;**30**(7):1575-1578

[94] Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G. Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Experimental Neurology. 2007;**207**(2):267-274

[95] Keilhoff G, Goihl A, Langnase K, Fansa H, Wolf G. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. European Journal of Cell Biology. 2006;**85**(1):11-24

[96] Zaminy A, Shokrgozar MA, Sadeghi Y, Noroozian M, Heidari MH, Piryaei A. Mesenchymal stem cells as an alternative for Schwann cells in rat spinal cord injury. Iranian Biomedical Journal. 2013;**17**(3):113

[97] Cj P, Tong L, Ll J, Wang Z, Zhang X, Gao H, et al. Synergistic effects of ultrashort wave and bone marrow stromal cells on nerve regeneration with acellular nerve allografts. Synapse. 2013;**67**(10):637-647

[98] Zhao Z, Wang Y, Peng J, Ren Z, Zhang L, Guo Q, et al. Improvement in nerve regeneration through a decellularized nerve graft by supplementation with bone marrow stromal cells in fibrin. Cell Transplantation. 2014;**23**(1):97-110

[99] Shalaby SM, Amal S, Ahmed FE, Shaban SF, Wahdan RA, Kandel WA, et al. Combined Wharton's jelly derived mesenchymal stem cells and nerve guidance conduit: A potential promising therapy for peripheral nerve injuries. The International Journal of Biochemistry & Cell Biology. 2017;**86**:67-76

[100] Schmidt CE, Leach JB. Neural tissue engineering: Strategies for repair and regeneration. Annual Review of Biomedical Engineering. 2003;**5**(1):293-347

[101] Tabesh H, Amoabediny G, Nik NS, Heydari M, Yosefifard M, Siadat SR, et al. The role of biodegradable engineered scaffolds seeded with Schwann cells for spinal cord regeneration. Neurochemistry International. 2009;**54**(2):73-83

[102] Deshmukh SN, Dive AM, Moharil R, Munde P. Enigmatic insight into collagen. Journal of Oral and Maxillofacial Pathology: JOMFP. 2016;**20**(2):276

[103] Brown R, Alovskaya A, Alekseeva T, Phillips J, King V. Fibronectin, collagen, fibrincompnents of extracellular matrix for nerve regeneration. In: Topics in Tissue Engineering. Vol. 3. Finland: Oulu University; 2007. pp. 1-26

[104] Amado S, Rodrigues JM, Luís AL, Armada-da-Silva PA, Vieira M, Gartner A, et al. Effects of collagen membranes enriched with in vitro-differentiated N1E-115 cells on rat sciatic nerve regeneration after end-to-end repair. Journal of Neuroengineering and Rehabilitation. 2010;**7**(1):7

[105] Brown RA, Phillips JB. Cell responses to biomimetic protein scaffolds used in tissue repair and engineering. International Review of Cytology. 2007;**262**:75-150

[106] Yoshii S, Oka M. Peripheral nerve regeneration along collagen filaments. Brain Research. 2001;**888**(1):158-162

[107] Phillips JB, Bunting SC, Hall SM, Brown RA. Neural tissue engineering: A self-organizing collagen guidance conduit. Tissue Engineering. 2005;**11**(9-10):1611-1617

[108] Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G, Kennedy WR, Tranquillo RT. Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. Experimental Neurology. 1999;**158**(2):290-300

[109] Chamberlain LJ, Yannas IV, Hsu HP, Spector M. Connective tissue response to tubular implants for peripheral nerve regeneration: The role of myofibroblasts. Journal of Comparative Neurology. 2000;**417**(4):415-430

[110] Archibald SJ, Krarup C, Shefner J, Li ST, Madison RD. A collagen-based nerve guide conduit for peripheral nerve repair: An electrophysiological study of nerve regeneration in rodents and nonhuman primates. Journal of Comparative Neurology. 1991;**306**(4):685-696

[111] Luís A, Rodrigues J, Geuna S, Amado S, Simões M, Fregnan F, et al. Neural cell transplantation effects on sciatic nerve regeneration after a standardized crush injury in the rat. Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery. 2008;**28**(6):458-470

[112] Mackinnon SE, Hudson AR, Bojanowski V, Hunter DA, Maraghi E. Peripheral nerve injection injury with purified bovine collagen—An experimental model in the rat. Annals of Plastic Surgery. 1985;**14**(5):428-436

[113] Kramer BA, Kadar AG, Clark K. Use of the Neuro-Wrap system for severe post-electroconvulsive therapy headaches. The Journal of ECT. 2008;**24**(2):152-155

[114] Li S-T, Yuen D. Implant devices for nerve repair. Google Patents; 2004

[115] Lohmeyer JA, Siemers F, Machens H-G, Mailänder P. The clinical use of artificial nerve conduits for digital nerve repair: A prospective cohort study and literature review. Journal of Reconstructive Microsurgery. 2009;**25**(01):055-061

[116] Salvatore L, Madaghiele M, Parisi C, Gatti F, Sannino A. Crosslinking of micropatterned collagen-based nerve guides to modulate the expected half-life. Journal of Biomedical Materials Research Part A. 2014;**102**(12):4406-4414

[117] Yang CR, Di Chen J. Preparation and biological evaluation of chitosan–collagen–icariin composite scaffolds for neuronal regeneration. Neurological Sciences. 2013;**34**(6):941-947

[118] Cerri F, Salvatore L, Memon D, Boneschi FM, Madaghiele M, Brambilla P, et al. Peripheral nerve morphogenesis induced by scaffold micropatterning. Biomaterials. 2014;**35**(13):4035-4045

[119] Bąk M, Gutkowska O, Wagner E, Gosk J. The role of chitin and chitosan in peripheral nerve reconstruction. Polimery w Medycynie. 2017;**47**(1):43-47

[120] Lu G, Kong L, Sheng B, Wang G, Gong Y, Zhang X. Degradation of covalently cross-linked carboxymethyl chitosan and its potential application for peripheral nerve regeneration. European Polymer Journal. 2007;**43**(9):3807-3818

[121] Freier T, Koh HS, Kazazian K, Shoichet MS. Controlling cell adhesion and degradation of chitosan films by N-acetylation. Biomaterials. 2005;**26**(29):5872-5878

[122] Freier T, Montenegro R, Koh HS, Shoichet MS. Chitin-based tubes for tissue engineering in the nervous system. Biomaterials. 2005;**26**(22):4624-4632

[123] Wang W, Itoh S, Matsuda A, Ichinose S, Shinomiya K, Hata Y, et al. Influences of mechanical properties and permeability on chitosan nano/microfiber mesh tubes as a scaffold for nerve regeneration. Journal of Biomedical Materials Research Part A. 2008;**84**(2):557-566

[124] Shirosaki Y, Hayakawa S, Osaka A, Lopes MA, Santos JD, Geuna S, et al. Challenges for nerve repair using chitosan-siloxane hybrid porous scaffolds. BioMed Research

International. 2014;**2014**:1-7. DOI: 10.1155/2014/153808

[125] Wang W, Itoh S, Matsuda A, Aizawa T, Demura M, Ichinose S, et al. Enhanced nerve regeneration through a bilayered chitosan tube: The effect of introduction of glycine spacer into the CYIGSR sequence. Journal of Biomedical Materials Research Part A: An Official Journal of the Society for Biomaterials, The Japanese Society for Biomaterials, and the Australian Society for Biomaterials and the Korean Society for Biomaterials. 2008;**85**(4):919-928

[126] Szymańska E, Winnicka K. Stability of chitosan—A challenge for pharmaceutical and biomedical applications. Marine Drugs. 2015;**13**(4):1819-1846

[127] Mingyu C, Kai G, Jiamou L, Yandao G, Nanming Z, Xiufang Z. Surface modification and characterization of chitosan film blended with poly-L-lysine. Journal of Biomaterials Applications. 2004;**19**(1):59-75

[128] Cheng M, Deng J, Yang F, Gong Y, Zhao N, Zhang X. Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. Biomaterials. 2003;**24**(17):2871-2880

[129] Wang X, Hu W, Cao Y, Yao J, Wu J, Gu X. Dog sciatic nerve regeneration across a 30-mm defect bridged by a chitosan/PGA artificial nerve graft. Brain. 2005;**128**(8):1897-1910

[130] Gärtner A, Pereira T, Simões MJ, Armada-da-Silva PA, França ML, Sousa R, et al. Use of hybrid chitosan membranes and human mesenchymal stem cells from the Wharton jelly of umbilical cord for promoting nerve regeneration in an axonotmesis rat model. Neural Regeneration Research. 2012;**7**(29):2247

[131] Itoh S, Suzuki M, Yamaguchi I, Takakuda K, Kobayashi H, Shinomiya K, et al. Development of a nerve scaffold using a tendon chitosan tube. Artificial Organs. 2003;**27**(12):1079-1088

[132] Lin Y-L, Jen J-C, Hsu S-H, Chiu M. Sciatic nerve repair by microgrooved nerve conduits made of chitosan-gold nanocomposites. Surgical Neurology. 2008;**70**:S9-S18

[133] Haastert-Talini K, Geuna S, Dahlin LB, Meyer C, Stenberg L, Freier T, et al. Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. Biomaterials. 2013;**34**(38):9886-9904

[134] Gonzalez-Perez F, Cobianchi S, Geuna S, Barwig C, Freier T, Udina E, et al. Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat. Microsurgery. 2015;**35**(4):300-308

[135] Baldrick P. The safety of chitosan as a pharmaceutical excipient. Regulatory Toxicology and Pharmacology. 2010;**56**(3):290-299

[136] Patel M, Mao L, Wu B, VandeVord PJ. GDNF–chitosan blended nerve guides: A functional study. Journal of Tissue Engineering and Regenerative Medicine. 2007;**1**(5):360-367

[137] Boucher TJ, Okuse K, Bennett DL, Munson JB, Wood JN, McMahon SB. Potent analgesic effects of GDNF in neuropathic pain states. Science. 2000;**290**(5489):124-127

[138] Hsu S-H, Kuo W-C, Chen Y-T, Yen C-T, Chen Y-F, Chen K-S, et al. New nerve regeneration strategy combining laminin-coated chitosan conduits and stem cell therapy. Acta Biomaterialia. 2013;**9**(5):6606-6615

[139] Lauto A, Foster LJ, Avolio A, Sampson D, Raston C, Sarris M, et al.

Sutureless nerve repair with laser-activated chitosan adhesive: A pilot in vivo study. Photomedicine and Laser Surgery. 2008;**26**(3):227-234

[140] Cortez P, Shirosaki Y, Botelho C, Simões M, Gartner F, da Costa R, et al. Hybrid chitosan membranes tested in sheep for guided tissue regeneration. In: Key Engineering Materials. Vols. 361-363. Switzerland: Trans Tech Publications; 2008. pp. 1265-1268. DOI: 10.4028/www.scientific.net/KEM.361-363.1265

[141] Shirosaki Y, Hayakawa S, Osaka A, Santos JD, Maurício AC. Nerve regeneration by using of chitosansilicate hybrid porous membranes. In: Key Engineering Materials. Vols. 529-530(1). Switzerland: Trans Tech Publications; 2013. pp. 361-364. DOI: 10.4028/www.scientific.net/KEM.529-530.361

[142] Shirosaki Y, Tsuru K, Hayakawa S, Osaka A, Lopes MA, Santos JD, et al. In vitro cytocompatibility of MG63 cells on chitosan-organosiloxane hybrid membranes. Biomaterials. 2005;**26**(5):485-493

[143] Shirosaki Y, Tsuru K, Hayakawa S, Osaka A, Lopes MA, Santos JD, et al. Physical, chemical and in vitro biological profile of chitosan hybrid membrane as a function of organosiloxane concentration. Acta Biomaterialia. 2009;**5**(1):346-355

[144] Amado S, Simoes M, da Silva PA, Luís A, Shirosaki Y, Lopes M, et al. Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. Biomaterials. 2008;**29**(33):4409-4419

[145] Tateishi T, Chen G, Ushida T. Biodegradable porous scaffolds for tissue engineering. Journal of Artificial Organs. 2002;**5**(2):77-83

[146] Shirosaki Y, Okayama T, Tsuru K, Hayakawa S, Osaka A. Synthesis and cytocompatibility of porous chitosan–silicate hybrids for tissue engineering scaffold application. Chemical Engineering Journal. 2008;**137**(1):122-128

[147] Simoes M, Gärtner A, Shirosaki Y, da Costa RG, Cortez P, Gartner F, et al. In vitro and in vivo chitosan membranes testing for peripheral nerve reconstruction. Acta Medica Portuguesa. 2011;**24**(1):43-52

[148] Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2001;**55**(2):151-157

[149] Neubrech F, Heider S, Harhaus L, Bickert B, Kneser U, Kremer T. Chitosan nerve tube for primary repair of traumatic sensory nerve lesions of the hand without a gap: study protocol for a randomized controlled trial. Trials. 2016;17:48. DOI: 10.1186/s13063-015-1148-5

[150] Fornasari BE, Gambarotta G, Ronchi G, Raimondo S, Crosio A, Budau CA, et al. Chitosan tubes enriched by skeletal muscle for peripheral nerve regeneration. In: 6th Symposium on Surgery of Peripheral Nerves. 2017

[151] Willerth SM, Sakiyama-Elbert SE. Approaches to neural tissue engineering using scaffolds for drug delivery. Advanced Drug Delivery Reviews. 2007;**59**(4-5):325-338

[152] Dellon A, Chang B. An alternative incision for approaching recurrent

median nerve compression at the wrist. Plastic and Reconstructive Surgery. 1992;**89**(3):576-578

[153] Costa HJZR, Bento RF, Salomone R, Azzi-Nogueira D, Zanatta DB, Costa MP, et al. Mesenchymal bone marrow stem cells within polyglycolic acid tube observed in vivo after six weeks enhance facial nerve regeneration. Brain Research. 2013;**1510**:10-21

[154] Arslantunali D, Dursun T, Yucel D, Hasirci N, Hasirci V. Peripheral nerve conduits: Technology update. Medical Devices (Auckland, NZ). 2014;**7**:405

[155] Zhang Z, Ortiz O, Goyal R, Kohn J. Biodegradable polymers. In: Principles of Tissue Engineering. Netherlands: Elsevier; 2014. pp. 441-473. DOI: 10.1016/B978-0-12-398358-9.00023-9

[156] Lu M-C, Huang Y-T, Lin J-H, Yao C-H, Lou C-W, Tsai C-C, et al. Evaluation of a multi-layer microbraided polylactic acid fiber-reinforced conduit for peripheral nerve regeneration. Journal of Materials Science: Materials in Medicine. 2009;**20**(5):1175-1180

[157] S-h H, Chan S-H, Chiang C-M, Chen CC-C, Jiang C-F. Peripheral nerve regeneration using a microporous polylactic acid asymmetric conduit in a rabbit long-gap sciatic nerve transection model. Biomaterials. 2011;**32**(15):3764-3775

[158] Matsumine H, Sasaki R, Yamato M, Okano T, Sakurai H. A polylactic acid non-woven nerve conduit for facial nerve regeneration in rats. Journal of Tissue Engineering and Regenerative Medicine. 2014;**8**(6):454-462

[159] Ni H-C, Tseng T-C, Chen J-R, Hsu S-H, Chiu M. Fabrication of bioactive conduits containing the fibroblast growth factor 1 and neural stem cells for peripheral nerve regeneration across

a 15 mm critical gap. Biofabrication. 2013;**5**(3):035010

[160] Reed A, Gilding D. Biodegradable polymers for use in surgery—Poly (glycolic)/poly (Iactic acid) homo and copolymers: 2. In vitro degradation. Polymer. 1981;**22**(4):494-498

[161] Oh SH, Lee JH. Fabrication and characterization of hydrophilized porous PLGA nerve guide conduits by a modified immersion precipitation method. Journal of Biomedical Materials Research Part A. 2007;**80**(3):530-538

[162] Lin K-M, Shea J, Gale BK, Sant H, Larrabee P, Agarwal J. Nerve growth factor released from a novel PLGA nerve conduit can improve axon growth. Journal of Micromechanics and Microengineering. 2016;**26**(4):045016

[163] Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. Tissue Engineering. 2000;**6**(2):119-127

[164] Pereira T, Gärtner A, Amorim I, Almeida A, Caseiro A, Armadada-Silva PA, et al. Promoting nerve regeneration in a neurotmesis rat model using poly(DL-lactide-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly: In vitro and in vivo analysis. BioMed Research International. 2014;**2014**:1-17. DOI: 10.1155/2014/302659

[165] Labroo P, Shea J, Edwards K, Ho S, Davis B, Sant H, et al. Novel drug delivering conduit for peripheral nerve regeneration. Journal of Neural Engineering. 2017;**14**(6):066011

[166] Doubra N, Amiri A, Jamalpoor Z, Fooladi AAI, Nourani MR. Fabrication of PLGA conduit for peripheral nerve regeneration. Journal of Applied Tissue Engineering. 2014;**1**(1):13-19

[167] Venugopal J, Zhang Y, Ramakrishna S. Electrospun nanofibres: Biomedical applications. Proceedings of the Institution of Mechanical Engineers, Part N: Journal of Nanoengineering and Nanosystems. 2004;**218**(1):35-45

[168] Radulescu D, Dhar S, Young CM, Taylor DW, Trost H-J, Hayes DJ, et al. Tissue engineering scaffolds for nerve regeneration manufactured by ink-jet technology. Materials Science and Engineering: C. 2007;**27**(3):534-539

[169] McConnell MP, Dhar S, Nguyen T, Naran S, Calvert JW, Sundine MJ, et al. Nerve growth factor expression response to induction agent booster dosing in transfected human embryonic kidney cells. Plastic and Reconstructive Surgery. 2005;**115**(2):506-514

[170] Luis AL, Rodrigues JM, Amado S, Veloso AP, Armada-Da-silva PA, Raimondo S, et al. PLGA 90/10 and caprolactone biodegradable nerve guides for the reconstruction of the rat sciatic nerve. Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery. 2007;**27**(2):125-137

[171] Shin RH, Friedrich PF, Crum BA, Bishop AT, Shin AY. Treatment of a segmental nerve defect in the rat with use of bioabsorbable synthetic nerve conduits: A comparison of commercially available conduits. JBJS. 2009;**91**(9):2194-2204

[172] Chiriac S, Facca S, Diaconu M, Gouzou S, Liverneaux P. Experience of using the bioresorbable copolyester poly (DL-lactide-ε-caprolactone) nerve conduit guide Neurolac™ for nerve repair in peripheral nerve defects: Report on a series of 28 lesions. Journal of Hand Surgery (European Volume). 2012;**37**(4):342-349

[173] Grant C, Twigg P, Egan A, Moody A, Smith A, Eagland D, et al. Poly(vinyl alcohol) hydrogel as a biocompatible viscoelastic mimetic for articular cartilage. Biotechnology Progress. 2006;**22**(5):1400-1406

[174] Alexandre N, Amorim I, Caseiro AR, Pereira T, Alvites R, Rêma A, et al. Long term performance evaluation of small-diameter vascular grafts based on polyvinyl alcohol hydrogel and dextran and MSCs-based therapies using the ovine pre-clinical animal model. International Journal of Pharmaceutics. 2017;**523**(2):515-530

[175] Alexandre N, Costa E, Coimbra S, Silva A, Lopes A, Rodrigues M, et al. In vitro and in vivo evaluation of blood coagulation activation of polyvinyl alcohol hydrogel plus dextran-based vascular grafts. Journal of Biomedical Materials Research Part A. 2015;**103**(4):1366-1379

[176] Bichara DA, Zhao X, Hwang NS, Bodugoz-Senturk H, Yaremchuk MJ, Randolph MA, et al. Porous poly (vinyl alcohol)-alginate gel hybrid construct for neocartilage formation using human nasoseptal cells. Journal of Surgical Research. 2010;**163**(2):331-336

[177] Rutkowski GE, Heath CA. Development of a bioartificial nerve graft. II. Nerve regeneration in vitro. Biotechnology Progress. 2002;**18**(2):373-379

[178] Ribeiro J, Caseiro AR, Pereira T, Armada-da-Silva PA, Pires I, Prada J, et al. Evaluation of PVA biodegradable electric conductive membranes for nerve regeneration in axonotmesis injuries: The rat sciatic nerve animal model. Journal of Biomedical Materials Research Part A. 2017;**105**(5):1267-1280

[179] Ribeiro J, Pereira T, Caseiro AR, Armada-da-Silva P, Pires I, Prada J, et al.

Evaluation of biodegradable electric conductive tube-guides and mesenchymal stem cells. World Journal of Stem Cells. 2015;**7**(6):956

[180] Chen Z-L, Yu W-M, Strickland S. Peripheral regeneration. Annual Review of Neuroscience. 2007;**30**:209-233

[181] Amano T, Richelson E, Nirenberg M. Neurotransmitter synthesis by neuroblastoma clones. Proceedings of the National Academy of Sciences. 1972;**69**(1):258-263

[182] Kimhi Y, Palfrey C, Spector I, Barak Y, Littauer U. Maturation of neuroblastoma cells in the presence of dimethylsulfoxide. Proceedings of the National Academy of Sciences. 1976;**73**(2):462-466

[183] Prasad KN, Kentroti S, Edwards-Prasad J, Vernadakis A, Imam M, Carvalho E, et al. Modification of the expression of adenosine 3′,5′-cyclic monophosphate-induced differentiated functions in neuroblastoma cells by beta-carotene and D-alpha-tocopheryl succinate. Journal of the American College of Nutrition. 1994;**13**(3):298-303

[184] Rodrigues J, Luís A, Lobato J, Pinto M, Lopes M, Freitas M, et al. Determination of the intracellular Ca^{2+} concentration in the N1E-115 neuronal cell line in perspective of its use for peripheric nerve regeneration. Bio-medical Materials and Engineering. 2005;**15**(6):455-465

[185] Rodrigues J, Luís A, Lobato J, Pinto M, Faustino A, Hussain NS, et al. Intracellular Ca^{2+} concentration in the N1E-115 neuronal cell line and its use for peripheric nerve regeneration. Acta Médica Portuguesa. 2005;**18**(5):323-328

[186] Luís AL, Rodrigues JM, Geuna S, Amado S, Shirosaki Y, Lee JM, et al. Use of PLGA 90: 10 scaffolds enriched with in vitro–differentiated neural cells for

repairing rat sciatic nerve defects. Tissue Engineering Part A. 2008;**14**(6):979-993

[187] Fairbairn N, Randolph M, Redmond R. The clinical applications of human amnion in plastic surgery. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2014;**67**(5):662-675

[188] Fu YS, Cheng YC, Lin MYA, Cheng H, Chu PM, Chou SC, et al. Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: Potential therapeutic application for Parkinsonism. Stem Cells. 2006;**24**(1):115-124

[189] Frausin S, Viventi S, Falzacappa LV, Quattromani MJ, Leanza G, Tommasini A, et al. Wharton's jelly derived mesenchymal stromal cells: Biological properties, induction of neuronal phenotype and current applications in neurodegeneration research. Acta Histochemica. 2015;**117**(4-5):329-338

[190] Gärtner A, Pereira T, Armada-da-Silva P, Amorim I, Gomes R, Ribeiro J, et al. Use of poly(DL-lactide-ε-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly of the umbilical cord for promoting nerve regeneration in axonotmesis: In vitro and in vivo analysis. Differentiation. 2012;**84**(5):355-365

[191] Cheng L-N, Duan X-H, Zhong X-M, Guo R-M, Zhang F, Zhou C-P, et al. Transplanted neural stem cells promote nerve regeneration in acute peripheral nerve traction injury: Assessment using MRI. American Journal of Roentgenology. 2011;**196**(6):1381-1387

[192] Jiang L, Jones S, Jia X. Stem cell transplantation for peripheral nerve regeneration: Current options and opportunities. International Journal of Molecular Sciences. 2017;**18**(1):94

[193] Caseiro AR, Pereira T, Ribeiro J, Amorim I, Faria F, Bártolo PJ, et al. Neuro-muscular regeneration using scaffolds with mesenchymal stem cells (MSCs) Isolated from human umbilical cord Wharton's jelly: Functional and morphological analysis using rat sciatic nerve neurotmesis injury model. Procedia Engineering. 2015;**110**:106-113

[194] Marcus AJ, Woodbury D. Fetal stem cells from extra-embryonic tissues: Do not discard. Journal of Cellular and Molecular Medicine. 2008;**12**(3):730-742

[195] Peng J, Wang Y, Zhang L, Zhao B, Zhao Z, Chen J, et al. Human umbilical cord Wharton's jelly-derived mesenchymal stem cells differentiate into a Schwann-cell phenotype and promote neurite outgrowth in vitro. Brain Research Bulletin. 2011;**84**(3):235-243

[196] Ribeiro J, Pereira T, Amorim I, Caseiro AR, Lopes MA, Lima J, et al. Cell therapy with human MSCs isolated from the umbilical cord Wharton jelly associated to a PVA membrane in the treatment of chronic skin wounds. International Journal of Medical Sciences. 2014;**11**(10):979

[197] Pereira T, Armada-da Silva P, Amorim I, Rêma A, Caseiro A, Gärtner A, et al. Effects of human mesenchymal stem cells isolated from Wharton's jelly of the umbilical cord and conditioned media on skeletal muscle regeneration using a myectomy model. Stem Cells International. 2014;**2014**:1-16. DOI: 10.1155/2014/376918

[198] Gärtner A, Pereira T, Armada-da-Silva P, Amado S, Veloso A, Amorim I, et al. Effects of umbilical cord tissue mesenchymal stem cells (UCX®) on rat sciatic nerve regeneration after neurotmesis injuries. Journal of Stem Cells & Regenerative Medicine. 2014;**10**(1):14

[199] Luo L, He Y, Wang X, Key B, Lee BH, Li H, et al. Potential roles of dental pulp stem cells in neural regeneration and repair. Stem Cells International. 2018;**2018**:1-15. DOI: 10.1155/2018/1731289

[200] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences. 2000;**97**(25):13625-13630

[201] Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: A review. The Open Dentistry Journal. 2010;**4**:37

[202] Karbanová J, Soukup T, Suchánek J, Pytlík R, Corbeil D, Mokrý J. Characterization of dental pulp stem cells from impacted third molars cultured in low serum-containing medium. Cells Tissues Organs. 2011;**193**(6):344-365

[203] Gronthos S, Brahim J, Li W, Fisher L, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. Journal of Dental Research. 2002;**81**(8):531-535

[204] Kawashima N. Characterisation of dental pulp stem cells: A new horizon for tissue regeneration? Archives of Oral Biology. 2012;**57**(11):1439-1458

[205] Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. The Journal of Clinical Investigation. 2012;**122**(1):80-90

[206] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. Proceedings of

the National Academy of Sciences. 2003;**100**(10):5807-5812

[207] Kiraly M, Porcsalmy B, Pataki A, Kadar K, Jelitai M, Molnar B, et al. Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochemistry International. 2009;**55**(5):323-332

[208] Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, et al. Peripheral nerve regeneration by secretomes of stem cells from human exfoliated deciduous teeth. Stem Cells and Development. 2015;**24**(22):2687-2699

[209] Askari N, Yaghoobi M, Shamsara M, Esmaeili-Mahani S. Tetracycline-regulated expression of OLIG2 gene in human dental pulp stem cells lead to mouse sciatic nerve regeneration upon transplantation. Neuroscience. 2015;**305**:197-208

[210] Geng YW, Zhang Z, Liu MY, Hu WP. Differentiation of human dental pulp stem cells into neuronal by resveratrol. Cell Biology International. 2017;**41**(12):1391-1398

[211] Arthur A, Shi S, Zannettino AC, Fujii N, Gronthos S, Koblar SA. Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells. 2009;**27**(9):2229-2237

[212] Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, et al. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. The FASEB Journal. 2014;**28**(4):1634-1643

[213] Yamamoto T, Osako Y, Ito M, Murakami M, Hayashi Y, Horibe H, et al. Trophic effects of dental pulp stem cells on schwann cells in

peripheral nerve regeneration. Cell Transplantation. 2016;**25**(1):183-193

[214] Potdar PD, Jethmalani YD. Human dental pulp stem cells: Applications in future regenerative medicine. World Journal of Stem Cells. 2015;**7**(5):839

[215] Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, et al. Tubulation with dental pulp cells promotes facial nerve regeneration in rats. Tissue Engineering Part A. 2008;**14**(7):1141-1147

[216] Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, et al. PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. Journal of Tissue Engineering and Regenerative Medicine. 2011;**5**(10):823-830

[217] Sanen K, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J. Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: Potential for peripheral nerve repair? Journal of Tissue Engineering and Regenerative Medicine. 2017;**11**(12):3362-3372

[218] Dai L-G, Huang G-S, S-h H. Sciatic nerve regeneration by cocultured Schwann cells and stem cells on microporous nerve conduits. Cell Transplantation. 2013;**22**(11):2029-2039

[219] Ullah I, Park J-M, Kang Y-H, Byun J-H, Kim D-G, Kim J-H, et al. Transplantation of human dental pulp-derived stem cells or differentiated neuronal cells from human dental pulp-derived stem cells identically enhances regeneration of the injured peripheral nerve. Stem Cells and Development. 2017;**26**(17):1247-1257

[220] Omi M, Hata M, Nakamura N, Miyabe M, Kobayashi Y, Kamiya H, et al. Transplantation of dental pulp stem cells suppressed inflammation in sciatic nerves by promoting macrophage

polarization towards anti-inflammation phenotypes and ameliorated diabetic polyneuropathy. Journal of Diabetes Investigation. 2016;**7**(4):485-496

[221] Delorme B, Nivet E, Gaillard J, Häupl T, Ringe J, Devèze A, et al. The human nose harbors a niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic properties. Stem Cells and Development. 2009;**19**(6):853-866

[222] Rui K, Zhang Z, Tian J, Lin X, Wang X, Ma J, et al. Olfactory ecto-mesenchymal stem cells possess immunoregulatory function and suppress autoimmune arthritis. Cellular & Molecular Immunology. 2016;**13**(3):401

[223] Ercolin ACM, Roballo KCS, Casals JB, Pieri NCG, Souza AF, Barreto RSN, et al. Rabbit olfactory stem cells. Isolation protocol and characterization. Acta Cirurgica Brasileira. 2016;**31**(1):59-66

[224] Altunbaş K, Yaprakci MV, Celik S. Isolation and characterization of olfactory stem cells from canine olfactory mucosa. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2016;**22**(2):237-243. DOI: 10.9775/ kvfd.2015.14277

[225] Veron AD, Bienboire-Frosini C, Feron F, Codecasa E, Deveze A, Royer D, et al. Isolation and characterization of olfactory ecto-mesenchymal stem cells from eight mammalian genera. BMC Veterinary Research. 2018;**14**(1):17

[226] Tomé M, Lindsay SL, Riddell JS, Barnett SC. Identification of nonepithelial multipotent cells in the embryonic olfactory mucosa. Stem Cells. 2009;**27**(9):2196-2208

[227] Johnstone SA, Liley M, Dalby MJ, Barnett SC. Comparison of human olfactory and skeletal MSCs using osteogenic nanotopography to demonstrate bone-specific bioactivity of the surfaces. Acta Biomaterialia. 2015;**13**:266-276

[228] Lindsay SL, Johnstone SA, Mountford JC, Sheikh S, Allan DB, Clark L, et al. Human mesenchymal stem cells isolated from olfactory biopsies but not bone enhance CNS myelination in vitro. Glia. 2013;**61**(3):368-382

[229] Shafiee A, Kabiri M, Ahmadbeigi N, Yazdani SO, Mojtahed M, Amanpour S, et al. Nasal septum-derived multipotent progenitors: A potent source for stem cell-based regenerative medicine. Stem Cells and Development. 2011;**20**(12):2077-2091

[230] King NM, Perrin J. Ethical issues in stem cell research and therapy. Stem Cell Research & Therapy. 2014;**5**(4):85

[231] Marshall CT, Guo Z, Lu C, Klueber KM, Khalyfa A, Cooper NG, et al. Human adult olfactory neuroepithelial derived progenitors retain telomerase activity and lack apoptotic activity. Brain Research. 2005;**1045**(1-2):45-56

[232] Antonevich N, Hancharou A, Buschik O, Rydna A, Chekan V, Strinkevich E, et al. Human olfactory mucosa-derived mesenchymal stem cells suppress cytotoxic functions of CD8+ T-lymphocytes and natural killer cells. Journal of Allergy and Clinical Immunology. 2018;**141**(2):AB122

[233] Steinbach S, Proft F, Schulze-Koops H, Hundt W, Heinrich P, Schulz S, et al. Gustatory and olfactory function in rheumatoid arthritis. Scandinavian Journal of Rheumatology. 2011;**40**(3):169-177

[234] McDonald C, Mackay-Sim A, Crane D, Murrell W. Could cells from your nose fix your heart? Transplantation of olfactory stem cells in a rat model of

cardiac infarction. The Scientific World Journal. 2010;**10**:422-433

[235] Murrell W, Wetzig A, Donnellan M, Féron F, Burne T, Meedeniya A, et al. Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. Stem Cells. 2008;**26**(8):2183-2192

[236] Veron AD, Bienboire-Frosini C, Girard SD, Sadelli K, Stamegna J-C, Khrestchatisky M, et al. Syngeneic transplantation of olfactory ectomesenchymal stem cells restores learning and memory abilities in a rat model of global cerebral ischemia. Stem Cells International. 2018;**2018**:1-10. DOI: 10.1155/2018/2683969

[237] Nivet E, Vignes M, Girard SD, Pierrisnard C, Baril N, Devèze A, et al. Engraftment of human nasal olfactory stem cells restores neuroplasticity in mice with hippocampal lesions. The Journal of Clinical Investigation. 2011;**121**(7):2808-2820

[238] Bas E, Van De Water TR, Lumbreras V, Rajguru S, Goss G, Hare JM, et al. Adult human nasal mesenchymal-like stem cells restore cochlear spiral ganglion neurons after experimental lesion. Stem Cells and Development. 2013;**23**(5):502-514

[239] Pandit SR, Sullivan JM, Egger V, Borecki AA, Oleskevich S. Functional effects of adult human olfactory stem cells on early-onset sensorineural hearing loss. Stem Cells. 2011;**29**(4):670-677

[240] Young E, Westerberg B, Yanai A, Gregory-Evans K. The olfactory mucosa: A potential source of stem cells for hearing regeneration. Regenerative Medicine. 2018;**13**(05):581-593. DOI: 10.2217/rme-2018-0009

[241] Toft A, Tomé M, Lindsay SL, Barnett SC, Riddell JS. Transplant-mediated repair properties of

rat olfactory mucosal OM-I and OM-II sphere-forming cells. Journal of Neuroscience Research. 2012;**90**(3):619-631

[242] Xiao M, Klueber KM, Lu C, Guo Z, Marshall CT, Wang H, et al. Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery. Experimental Neurology. 2005;**194**(1):12-30

[243] Roche P, Alekseeva T, Widaa A, Ryan A, Matsiko A, Walsh M, et al. Olfactory derived stem cells delivered in a biphasic conduit promote peripheral nerve repair in vivo. Stem Cells Translational Medicine. 2017;**6**(10):1894-1904

[244] Kaplan HM, Mishra P, Kohn J. The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. Journal of Materials Science: Materials in Medicine. 2015;**26**(8):226

[245] Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, et al. Methods and protocols in peripheral nerve regeneration experimental research: Part I—Experimental models. International Review of Neurobiology. 2009;**87**:47-79

[246] Pavić R, Pavić ML, Tvrdeić A, Tot OK, Heffer M. Rat sciatic nerve crush injury and recovery tracked by plantar test and immunohistochemistry analysis. Collegium Antropologicum. 2011;**35**(1):93-100

[247] Nichols CM, Myckatyn TM, Rickman SR, Fox IK, Hadlock T, Mackinnon SE. Choosing the correct functional assay: A comprehensive assessment of functional tests in the rat. Behavioural Brain Research. 2005;**163**(2):143-158

[248] Ozturk C. Peripheral nerve surgery models sciatic nerve crush injury model.

In: Plastic and Reconstructive Surgery. London: Springer-Verlag; 2015. pp. 513-517. DOI: 10.1007/978-1-4471-6335-0_63

[249] Dellon A, Mackinnon S. Sciatic nerve regeneration in the rat. Validity of walking track assessment in the presence of chronic contractures. Microsurgery. 1989;**10**(3):220-225

[250] Hayashi A, Moradzadeh A, Hunter DA, Kawamura DH, Puppala VK, Tung TH, et al. Retrograde labeling in peripheral nerve research: It is not all black and white. Journal of Reconstructive Microsurgery. 2007;**23**(07):381-389

[251] Carriel V, Garzón I, Alaminos M, Cornelissen M. Histological assessment in peripheral nerve tissue engineering. Neural Regeneration Research. 2014;**9**(18):1657

[252] Navarro X, Udina E. Methods and protocols in peripheral nerve regeneration experimental research: Part III—Electrophysiological evaluation. International Review of Neurobiology. 2009;**87**:105-126

[253] Zeidenberg J, Burks SS, Jose J, Subhawong TK, Levi AD. The utility of ultrasound in the assessment of traumatic peripheral nerve lesions: Report of 4 cases. Neurosurgical Focus. 2015;**39**(3):E3

[254] Shen N, Zhu J. Application of sciatic functional index in nerve functional assessment. Microsurgery. 1995;**16**(8):552-555

[255] de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Experimental Neurology. 1982;**77**(3):634-643

[256] Algora J, Chen LE, Seaber AV, Wong GH, Urbaniak JR. Functional effects of lymphotoxin on crushed peripheral nerve. Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery. 1996;**17**(3):131-135

[257] Varejão AS, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, et al. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. Journal of Neurotrauma. 2004;**21**(11):1652-1670

[258] Dinh P, Hazel A, Palispis W, Suryadevara S, Gupta R. Functional assessment after sciatic nerve injury in a rat model. Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery. 2009;**29**(8):644-649

[259] Bain J, Mackinnon S, Hunter D. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plastic and Reconstructive Surgery. 1989;**83**(1):129-138

[260] Thalhammer J, Vladimirova M, Bershadsky B, Strichartz G. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. The Journal of the American Society of Anesthesiologists. 1995;**82**(4):1013-1025

[261] Koka R, Hadlock TA. Quantification of functional recovery following rat sciatic nerve transection. Experimental Neurology. 2001;**168**(1):192-195

[262] Wong K-H, Kanagasabapathy G, Bakar R, Phan C-W, Sabaratnam V. Restoration of sensory dysfunction following peripheral nerve injury by the polysaccharide from culinary and medicinal mushroom, Hericium erinaceus (Bull.: Fr.) Pers. through its neuroregenerative action. Food Science and Technology. 2015;**35**(4):712-721

[263] Boissé L, Spencer SJ, Mouihate A, Vergnolle N, Pittman QJ. Neonatal immune challenge alters nociception in the adult rat. Pain. 2005;**119**(1-3):133-141

[264] Hu D, Hu R, Berde CB. Neurologic evaluation of infant and adult rats before and after sciatic nerve blockade. Anesthesiology: The Journal of the American Society of Anesthesiologists. 1997;**86**(4):957-965

[265] Bozkurt A, Tholl S, Wehner S, Tank J, Cortese M, Mon O'Dey D, et al. Evaluation of functional nerve recovery with Visual-SSI—A novel computerized approach for the assessment of the static sciatic index (SSI). Journal of Neuroscience Methods. 2008;**170**(1):117-122

[266] Varejão AS, Cabrita AM, Meek MF, Bulas-Cruz J, Filipe VM, Gabriel RC, et al. Ankle kinematics to evaluate functional recovery in crushed rat sciatic nerve. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine. 2003;**27**(6):706-714

[267] Maurício AC, Gärtner A, Armada-da-Silva P, Amado S, Pereira T, Veloso ANP, et al. Cellular systems and biomaterials for nerve regeneration in neurotmesis injuries. In: Biomaterials Applications for Nanomedicine. London: Intechopen; 2011. pp. 415-440. DOI: 10.5772/24247

[268] Raimondo S, Fornaro M, Di Scipio F, Ronchi G, Giacobini-Robecchi MG, Geuna S. Methods and protocols in peripheral nerve regeneration experimental research: Part II—Morphological techniques. International Review of Neurobiology. 2009;**87**:81-103

[269] Bozkurt A, Lassner F, O'Dey D, Deumens R, Böcker A, Schwendt T, et al. The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves. Biomaterials. 2012;**33**(5):1363-1375

[270] Hirano A. The role of electron microscopy in neuropathology. Acta Neuropathologica. 2005;**109**(1):115-123

[271] Ronchi G, Jager SB, Vaegter CB, Raimondo S, Giacobini-Robecchi MG, Geuna S. Discrepancies in quantitative assessment of normal and regenerated peripheral nerve fibers between light and electron microscopy. Journal of the Peripheral Nervous System. 2014;**19**(3):224-233