Strategies to improve nerve regeneration after radical prostatectomy: a narrative review.

Strategies to improve nerve regeneration after radical prostatectomy: a narrative review

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Abstract

Peripheral nerves are complex organs that spread throughout the entire human body. They are frequently affected by lesions not only as a result of trauma but also following radical tumor resection. In fact, despite the advancement in surgical techniques, such as nerve-sparing robot assisted radical prostatectomy, some degree of nerve injury may occur resulting in erectile dysfunction with significant impairment of the quality of life.

The aim of this review is to provide an overview on the mechanisms of the regeneration of injured peripheral nerves and to describe the potential strategies to improve the regeneration process and the functional recovery. Yet, the recent advances in bio-engineering strategies to promote nerve regeneration in the urological field are outlined with a view on the possible future regenerative therapies which might ameliorate the functional outcome after radical prostatectomy.

Introduction

Radical prostatectomy is the gold standard surgical treatment for organ-confined prostate cancer. The employment of innovative surgical technique such as nerve-sparing robot assisted radical prostatectomy allowed to magnify the anatomical field leading to a three-dimensional perspective obtained through the robotic lenses and a better anatomical knowledge.

Despite surgical technique advancement, erectile dysfunction rate after radical prostatectomy is still high especially in minimal nerve-sparing technique where frequently iatrogenic damage to the autonomic peri-prostatic nerve bundles occurs[1-3].

Anatomically, prostate innervations is supplied by peripheral innervation identified in the pelvic plexus with a dense innervations network composed by many nerves difficult to identify as well the intramural ganglia, particularly dense at prostate capsule level and caudal prostate [4,5].

Concerning the peripheral nervous system, we have to discriminate between somatic and autonomic components that show anatomical and structural differences.

The somatic nervous system includes the sensory and motor nerves that innervate the limbs and body wall. Sensory nerve fibers derive from neurons located in the dorsal root ganglion and supply skin innervations. The motor neurons cell bodies are located in the anterior horn of the spinal cord and supply innervations to the skeletal muscle in which they release acetylcholine that stimulates the voluntary contraction.

The autonomic nervous system consists of two main divisions, the sympathetic and the parasympathetic that affects the peripheral transmission of the visceral organs through nerves and ganglia. The efferent pathway involves two neurons: the preganglionic and the post ganglionic neurons, preganglionic axons are myelinated with an organization that resembles the somatic nerves, while postganglionic axon are unmyelinated and organized in small diameter bundles surrounded by a single Schwann cell (type C fibers). The sympathetic is involved in responses that would be associated with fighting or fleeing, the parasympathetic is involved in energy conservation functions and increases bladder contractility, gastrointestinal motility and secretion [6,7].

In the parasympathetic nervous system preganglionic fibers are myelinated and arise from different cranial nerves and from the second to fourth sacral spinal nerves. Postganglionic parasympathetic fibers are usually unmyelinated and shorter than the sympathetic fibers, since the ganglia they synapse are in or near the visceral they supply.

The aim of this narrative review is to provide a brief description of the different types of injuries that can occur in the peripheral nerves, and to describe the different steps of the regeneration process.

Somatic nerve lesions are common injuries often caused by trauma or accident at work, for this reason most of the experimental studies described here are performed viewing these nervous components. Despite this, the bases provided in this work have allowed us to understand more in detail the therapeutic possibilities in case of autonomic nerve lesions, and in particular in case of damage of the peri-prostatic bundles after radical prostatectomy.

1. THE BIOLOGY OF NERVE INJURY AND REGENERATION

1.1 Effects of mechanical injury on peripheral nerve fibers

Peripheral nerves represent the main component of the peripheral nervous system forming a complex network that reach the whole body, making them particularly vulnerable to injuries.

Each nerve is a complex structure formed by cellular and tissue elements, and surrounded by three basic protective connective tissue layers: the epineurium that support and surround the whole nerve, the perineurium that surround each nerve fascicle and the endoneurium that protects the nerve fiber [8-10].

The smallest functional unit of a peripheral nerve is the nerve fiber, responsible of the motor and sensory impulse conduction. Anatomically, depending on the strategy adopted from Schwann cells to enclose axons, nerve fibers are distinguished in two subgroups: myelinated and unmyelinated. Myelinated nerve fibers consist of a single axon that is enveloped individually by a single Schwann cell.

The Schwann cell membrane wraps around the nerve fiber to form a multilaminated myelin sheath. In myelinated fibers axons are enveloped by a chain of Schwann cells, arranged in longitudinal sequences along the length of the axon. Between each Schwann cell, there is an interspace of unmyelinated axon known as “node of Ranvier” in which the axolemma is exposed to the extracellular space. This area allows to extracellular ions to reach the axon, inducing the saltatory conduction of the impulse along the nerve fiber [10,11]. On the contrary, unmyelinated nerve fibers are composed by a group of several axons enveloped by a single Schwann cell [11].

Peripheral nerve injuries are common conditions with broad ranging symptoms depending on the severity and nerves involved. If not properly treated, nerve traumas could lead to a sensory and motor function deficit. Indeed, despite the spontaneous regeneration potential of peripheral nerve fibers, clinical results of nerve injuries are still unsatisfactory, resulting in not complete functional recovery.

Peripheral nerve injuries can be described and classified using Seddon degree in *neuropraxia, axonotmesis and neurotmesis* [12]:

- *Neurapraxia* is a mild injury, characterized by local myelin damage. It may result from exposure to a wide range of conditions such as heat, cold, irradiation or electrical injuries, but it is most commonly due to mechanical stress, such as concussion, compression or traction injuries. Axon continuity is preserved, and the nerve does not undergo Wallerian degeneration. Paralysis of the innervated body part may occur, with possible atrophy due to disuse. The absence of lesion of nerve connective structures makes surgery unnecessary and recovery usually occurs within few days, up to a few weeks.
- *Axonotmesis* is a middle type of injury, in which peripheral axons are damaged or destroyed, but the connective tissue structures remain intact. The axon interruption is often the result of nerve pinching, crushing or prolonged pressure. The distal stump of the nerve undergoes Wallerian degeneration, but the subsequent axonal regrowth may occur along the intact endoneurial tubes. The proximal nerve stump undergoes retrograde degeneration. The transmission of pulses is compromised, as well as the functionality of the connected area of the body. The time of recovery depends upon the internal nerve disorganization as well as the distance of the injury site to the end organ. The absence of lesion of nerve connective structures makes surgery unnecessary.
- *Neurotmesis* is the most severe type of injury. The nerve is completely disrupted with loss of tissue continuity, and the connective tissue is severely damaged. Even in this case the proximal nerve stump undergoes retrograde degeneration, while the distal to Wallerian degeneration. Functional recovery does not easily occur because of the extent of endoneurial tube disruption. Nonetheless, a surgical approach is required to get a proper nerve fibers regeneration.

  A further classification provided by Sunderland in 1951 subdivided the injuries according to the discontinuity of the different connective tissue layers in 5 degree [13].
- Sunderland type 1 injury correspond to Seddon's *Neurapraxia* with conduction block and completely intact stroma*;*

- Sunderland type 2 injury corresponds to Seddon's axonotmesis represents a severe crush injury with disconnection of axons and Schwann cells sheath but preservation of all connective tissue layers, endoneurium, perineurium, and epineurium are still intact, but the axons are physiologically disrupted. Recovery can occur by axonal regrowth along endoneurial tubes, and complete functional recovery can be expected. The time for recovery depends on the level of injury, usually months.
- In Sunderland type 3 injury, the endoneurial layer is disconnected but the surrounding perineurium and epineurium are intact. Recovery is incomplete and depends upon how well the axons can cross the site of the lesion and find endoneurial tubes.
- In Sunderland type 4 only epineurium continuity is preserved, individual nerve fascicles are transected, and the continuity of the nerve trunk is maintained only by the surrounding epineurium. This type of injury requires surgical repair or reconstruction of the nerve.
- Sunderland type 5 injury is equivalent to Seddon's neurotmesis (complete nerve trasaction), also the epineurium is disconnected resulting in a complete nerve transaction and spontaneous recovery is negligible.

  Although Sunderland's classification provides a concise and anatomic description of nerve injury, the clinical utility of this system is debatable since nerves may undergo a combination of different degrees of injury.

  Therefore, in 1988 Susan E. Mackinnon and A. Lee Dellon described a 6th degree of nerve injury to address a mixed nerve injury. They use the term "neuroma in continuity" to describe a combination of the degrees of injuries per fascicle [14] (Fig.1).

  Time of recovery of peripheral nerve depends on the degree of injury and is summarized in Table1.

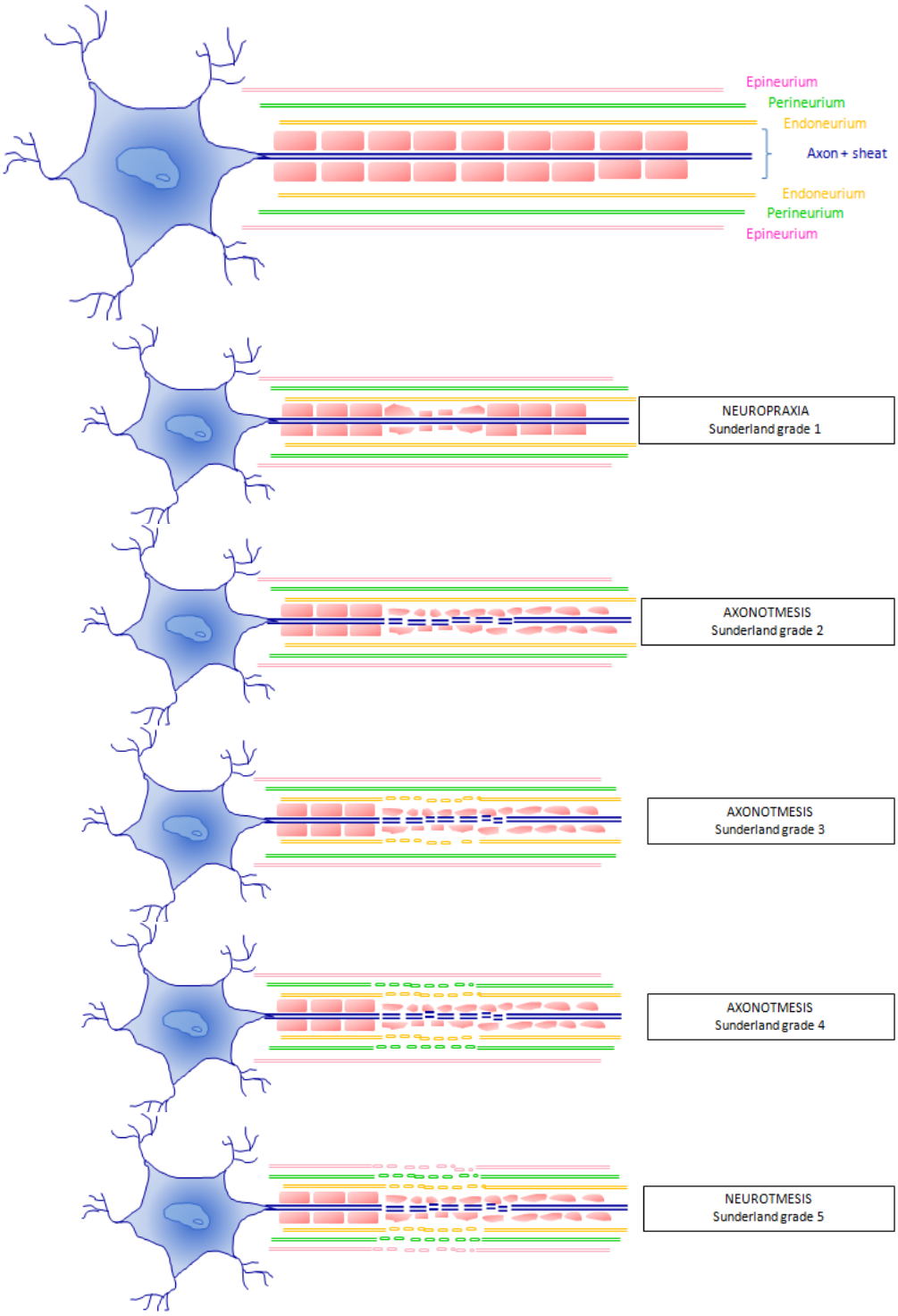

Fig.1: Schematic representation of nerve injuries

Degree of nerve injury	Spontaneous recovery	Time and rate of recovery	Surgery
First: Neuropraxia	Full	Fast: few days, occasionally few weeks	None
Second: Axonotmesis	Full/partial	Slow (1mm/day)	None
Third	Partial or incomplete	Slow (1mm/day)	None or neurolysis
Fourth	None	Regeneration occurs at rate of 1 inch/month	Nerve repair/Graft
Fifth: Neurotmesis	None	Regeneration occurs at rate of 1 inch/month	Nerve repair/Graft
Sixth: mixed injury	Recovery depends on the injury and the combination of different injury degree		

Table 1: Classification and time of recovery of different peripheral nerve injuries

1.2 The biological mechanisms of peripheral nerve regeneration

It is known that, differently to the Central Nervous System (CNS), the Peripheral Nervous System (PNS) is able to regenerate spontaneously in response to injury although the regeneration process is closely related to the severity of the damage. After a nerve injury, several mechanisms occur at the injury site almost immediately including morphologic and metabolic changes [15-17].

The interruption of a peripheral nerve causes significant changes in normal morphology and tissue organization both proximally and distally to the lesion site [17,18].

The functional significance of the regeneration process is to replace the distal nerve segment reaching the target organ and achieving the functional recovery.

After injury, axons distal to the lesion site are disconnected from the neuronal body and undergo degeneration called “Wallerian Degeneration” in honor of Augustus Volney Waller, who first characterized the disintegration of the frog glossopharyngeal and hypoglossal nerves after axotomy 160 years ago [19]. This process starts immediately after injury and involves myelin degradation of axoplasm, axolemma and myelin sheath due to proteolysis; myelin is transformed into neutral lipid compounds by Schwann cells, infiltrated blood monocytes and macrophages are recruited at the injury site [18,20]. Occurrence of Wallerian degeneration contributes to axonal regeneration due to clearance

of myelin debris and growth inhibitors, and subsequent creates a regenerative microenvironment favorable for the axonal regrowth of surviving neurons [21-24].

Whereas the Wallerian degeneration occurs, the soma reacts to the injury with substantial metabolic changes necessary for regeneration and axonal elongation. The most relevant morphological changes in the neuronal body are the Nissl bodies dissolution followed by nuclear eccentricity and enlargement, cell swelling, dendrites retraction.

In degeneration and regeneration Schwann cells play a critical role: during Wallerian degeneration an intense inflammatory response occurs, mediated by Schwann cells, mast cells, macrophages and activated endothelial cells leading an increase of chemokines, cytokines, interleukin 1 (IL-1) and tumor necrosis factor (TNF) [25,26].

Furthermore, Schwann cells begin to dedifferentiate in the distal stump: within 48 hours of injury, they change their gene expression: myelin proteins (e.g., P0, MAG) [27-29] and connexin 32 decrease dramatically as a consequence of axonal degeneration distal to the injury site, whereas regeneration-associated genes, and neurotrophin such as NGF, GDNF, BDNF and IGF, are up-regulated and promote axon growth [26,30-32].

Between days 1 and 5 after injury, Schwann cells start to proliferate peaking around day 3 post-injury and then decreasing during the following weeks. Proliferating Schwann cells align with the endoneurial tubes in columns known as bands of Büngner at the basal lamina level, that support and guide the regenerating axons; aligned Schwann cells and their extracellular matrix provide indispensable pathways to guide axonal regrowth [33-35].

Nerve regeneration and target reinnervation are complex processes, involving multiple factors. Even if the peripheral nervous system is able to regenerate, nerves can regenerate on their own if injuries are small; indeed, larger injuries must be surgically treated [36-38].

Regenerating axons are usually produced at the node of Ranvier located close to the proximal stump of the lesion [39,40] and extend by attaching themselves to the inner surface of the basal lamina or on the Schwann cell plasma membrane (Fig.2).

The advancement of regenerating axons in the distal segment is promoted by different factors such as fibronectin and laminin together with several cell adhesion molecules through the Schwann cell column[41].

Adhesion molecules are no longer detected at around the time that Schwann cells begin to form the myelin sheath on the axon, whereas the mature unmyelinated fibers in which the relationship between axons and Schwann cells is apparently preserved as that during the development, continue to exhibit such adhesion molecules [42].

Axons can sprout approaching Schwann cells column or randomly in the connective tissue of the nerve. After few time, regenerated axons that reached the target organs display a close-to-normal diameter, branches that do not reach the target are pruned away [43].

The knowledge of molecular and cellular changes occurring during the degeneration and the regeneration process of a peripheral nerve is of importance when attempting to improve the available strategies for nerve repair.

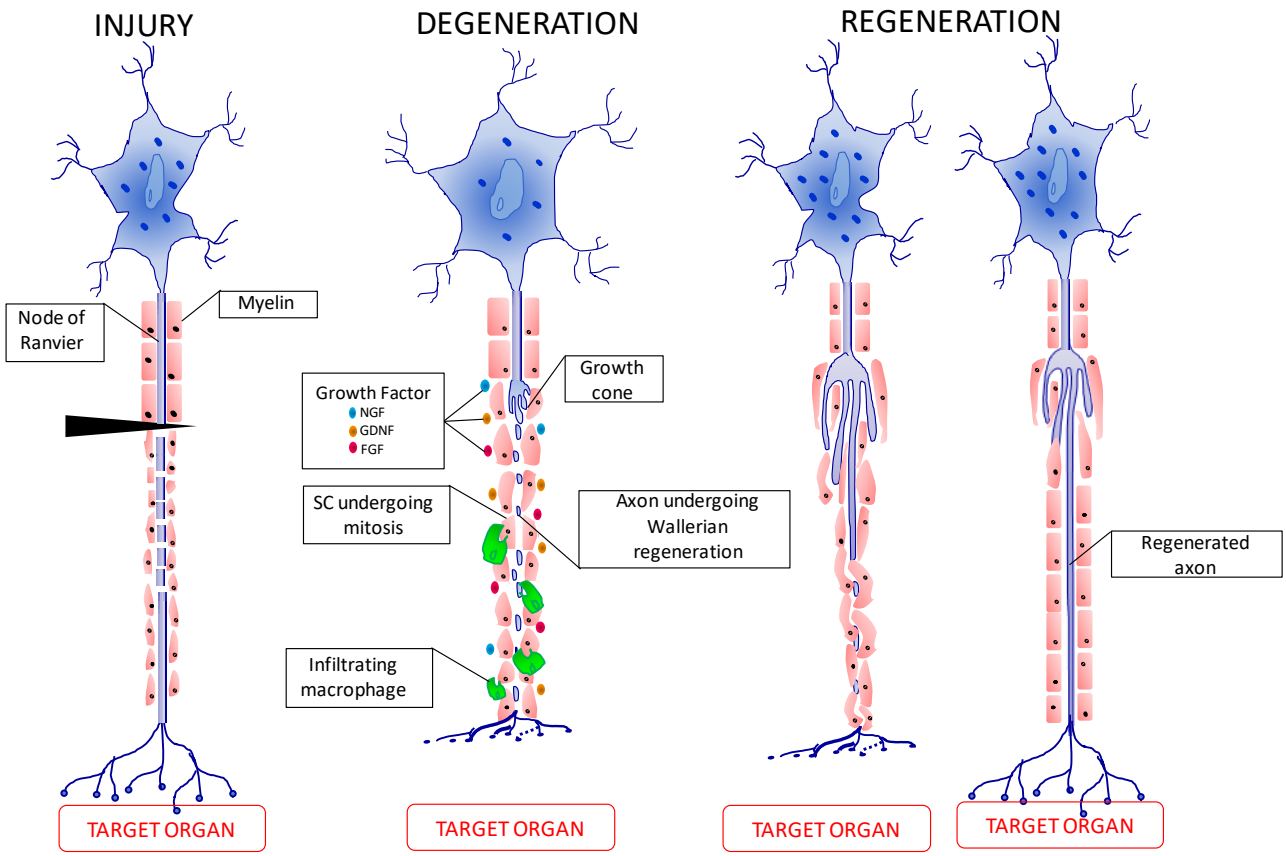

Fig. 2: Schematic representation of degeneration and regeneration processes

2. FACTORS INFLUENCING PERIPHERAL NERVE REGENERATION

In contrast to the central nervous system (CNS), the peripheral nervous system (PNS) is able to regenerate spontaneously after injury. The functional recovery in the PNS can be attributed to different factors, such as the ability of neurons and/or Schwann cells to regenerate neurites, the distal environment supporting axon regrowth, and the target tissues receptive to reinnervation [44]. In physiological conditions, nerve fibers regenerate after degeneration, and the successful nerve regeneration is considered closely linked to the Wallerian degeneration process.

Functional recovery after nerve injury depends on several extrinsic and intrinsic factors such as: surgeon experience, surgical technique used for the reconstruction, rehabilitation, obese conditions, co-morbidity and not least, age [45-47].

About co-morbidity, it is well know that axonal regeneration following peripheral nerve injury is impaired in diabetic patients and has been documented in experimental diabetic animal models: diabetic conditions induce alterations in the biological properties of neurons and Schwann cells leading to delay in Wallerian degeneration and macrophage infiltration, furthermore reduce the expression of neurotrophic factors and alteration of the extracellular matrix components resulting in impairment of axonal regeneration [48,49].

Age is a further factor that influences peripheral nerve regeneration. Whereas it is well reported that young tissue has high regeneration potential, in aged tissue the regenerative capability and functional recovery is significantly reduced. Results about the implantation of nerve conduit in aged rats showed that axonal regeneration after sciatic nerve defects was less effective in aged than in young mice when using either nerve autografts or nerve conduits [50].

Furthermore, morphological and morphometrical studies on crushed sciatic nerve of 6 and 24 month-old mice analyzed 2, 4, and 8 weeks after injury showed that two weeks after axotomy, fascicles of aged mice contained significantly fewer regenerated myelinated fibers than young; 4 weeks later, the difference in the number of myelinated fibers was less. However, measurements of myelinated fibers of aged mice showed that areas of Schwann cell cytoplasm and myelin were significantly reduced at all time points analyzed [51-55].

Since the aging population is progressively growing, more studies are needed to improve the regeneration process in aged animals.

3. STRATEGIES TO IMPROVE PERIPHERAL NERVE REGENERATION

Strategies to improve the regeneration of peripheral nerves are proposed to reach the functional recovery [56,57].

A possible approach is represented by the delivery of neurotrophic factor (NTFs) such as NGF, GDND and FGF into the nerve defect. It is known that neurotrophic factors support the different phases of Wallerian degeneration and axonal regeneration. For this reason, different studies report the application and the delivery of NTFs for instance conjugated with iron oxide nanoparticles (IONP), which were supposed to increase the stability of the conjugated NTFs, but also to ensure local and slow release of NTFs [58].

Another approach proposed to enhance nerve regeneration is the administration of drugs such as Sildenafil, a selective inhibitor of phosphodiesterase-5 (PDE5) causing intracellular accumulation of cGMP.

Sildenafil is currently used for treatment of erectile dysfunction, and other several conditions such as pulmonary hypertension [59,60].

Recently has been reported that increasing cGMP in cells can also induce neurogenesis, angiogenesis and synaptogenesis in young and old animal models promoting the functional recovery of sciatic nerve [61,62].

Different materials are currently investigated as device to enhance axonal regeneration, both of biologic or synthetic origin.

According to the material source, they can be classified in different categories: biological nerve conduits or syntethic nerve conduits.

3.1 Biomaterials currently used for peripheral nerve repair

Various materials have been used as gap for peripheral nerves and are classified in: non degradable materials, biodegradable synthetic material and biodegradable materials of natural origin.

Non degradable biomaterials

A wide range of synthetic non degradable materials have been studied and tested for the fabrication of artificial nerve device, they possess many useful chemical and physical properties such as the easy manipulation during the fabrication process.

The most common are represented by: silicone tubes**,** frequently used for nerve repairs empty or filled with collagen, laminin and fibronectin based gel. This material is neither biodegradable nor permeable to large molecules resulting in a possible fibrotic capsule formation around the guide.

Other non degradable materials employed for nerve regeneration are composed by elastomer hydrogel or pourus stainless steel with the disadvantage in strong scar tissue formation and foreign body reaction combined with inflexibility and lack of stability of the material [63,64].

Biodegradable synthetic materials

Among biodegradable material, the polyglycolic acid (PGA) represent a bioabsorbable material currently used as suture material for wound closure [65].

Polyesters and copolyesters have also been reported as suitable materials for nerve regeneration, to this category belong poly(L-lactide) (PLLA), poly(lactide-co-ε-caprolactone), poly(L-lactide-co-glycolide), poly(1,3-trimethylenecarbonate-co-ε-caprolactone) and poly(ε-caprolactone) (PCL) [66].

Biodegradable materials of natural origin

These materials are able to degrade within a reasonable period proving different useful properties such as flexibility, degradability, porosity and high biocompatibility.

Furthermore these important features can be modified altering the chemical or engineering properties of the materials.

Among these collagen, the major component of the extracellular matrix, has high biologic properties for peripheral nerve regeneration able to enhance the regeneration process but despite the successful results in animal experimental model, no clinical trials have been conducted in human to date [67-70].

Silk fibroin derived from natural silk has an increased application in biomedical fields thanks to its unique properties such as high tensile strength, elasticity and low immunogenicity [71]. In peripheral nerve regeneration, silk fibroin has been used as biomaterial for nerve guides to clarify the biocompatibility with neural tissues *in vitro*, and for bridging nerve defects *in vivo* [72-74].

Other natural proteins like keratins have also been tested as nerve scaffold materials [75,76] or as filler to support peripheral nerve regeneration [77].

Among the biodegradable materials chitosan, a copolymer of D-glucosamine and N-acetyl-D-glucosamine, obtained from full or partial N-deacetylation of chitin, represents a highly biocompatible, biodegradable, low toxic material. The reasonable of chitosan as a eligible biomaterial for the development of nerve guides resides in its favorable biological properties and in its ability to interact with the ECM molecules. The biocompatibility of chitosan-based biomaterials with CNS and PNS cells has been widely investigated with success [78-81]. Recently, the study of chitosan tubes alone or in combination with other proteins such as poly-L-lysine or with PGA demonstrated the efficiency of such nerve guides for bridging peripheral nerve defects [82-87].

Chitosan is a biodegradable scaffold with very positive effects on nerve regeneration, facilitating nerve healing and improving nerve growth.

For these reasons, chitosan is actually used as scaffold for regeneration of various tissues (nerve, skin, bone, cartilage) [85] (Fig. 3).

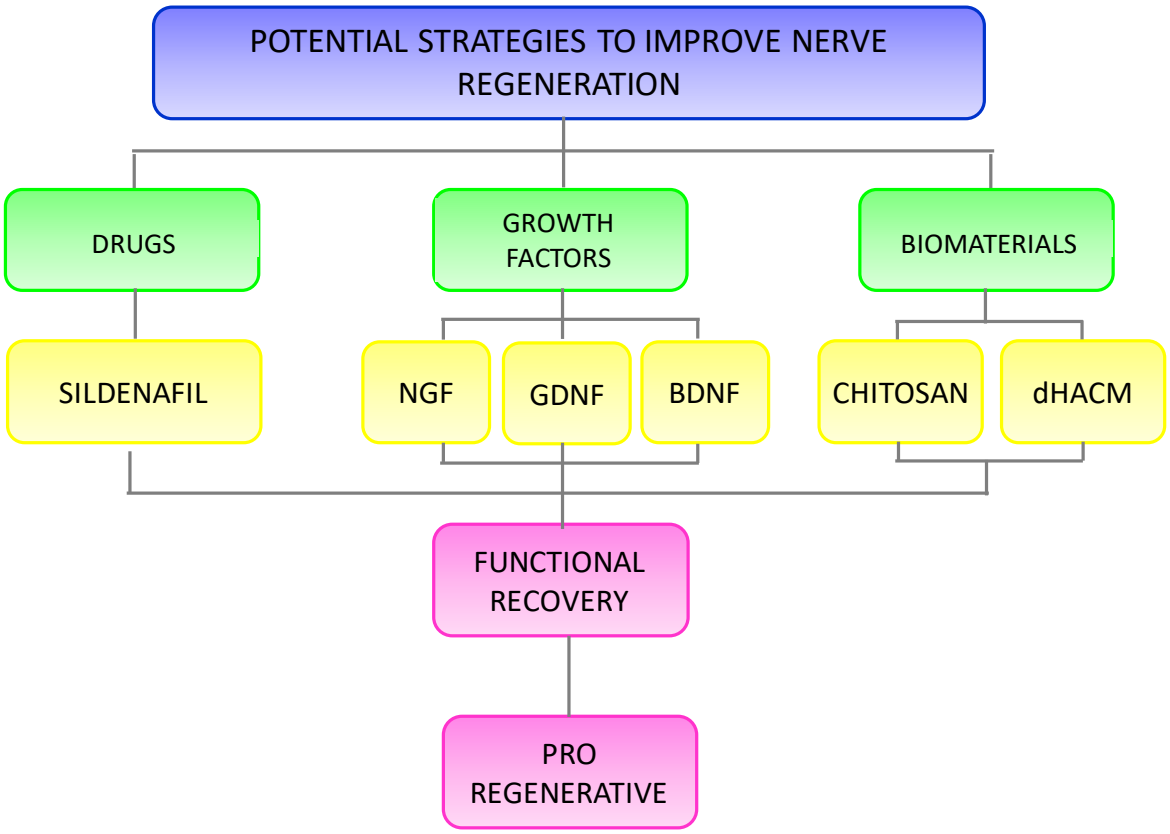

Fig. 3: Schematic representation of the potential strategies to improve nerve regeneration.

4. APPLICATION OF MEMBRANES TO IMPROVE THE REGENERATION OF PERI-PROSTATIC NERVES AFTER NERVE-SPARING ROBOT ASSISTED RADICAL PROSTATECTOMY

Recently, new strategies to improve the regeneration of the prostate nerves are arising to reach the functional recovery in patients who underwent radical prostatectomy. In urological context, the application of different type of membranes is particularly useful to protect and to enhance the regeneration process of the periprostatic nerve inside the neurovascular bundle that surround the prostate. In the following paragraphs a description of the two main clinical membranes applied to neurovascular bundle will be provide.

4.1 Application of dehydrated human amnion/chorion membrane on the neurovascular bundle after nerve sparing radical prostatectomy

Some authors described the application of dehydrated human amnion/chorion membranes (dHACM) on the neurovascular bundles after nerve sparing radical prostatectomy as a neuroprotective, pro-regenerative and anti-inflammatory device.

Patel et al. reported the clinical use of dHACM as a novel source of implantable neurotrophic factors and cytokines useful to enhance the regeneration process [88,89]. This pivotal study was conducted in patients who underwent bilateral nerve sparing radical prostatectomy with the bilateral application of dHACM for a total of 58 patients preoperatively potent and continent. Postoperative evaluation of patients by follow-up information showed that a high percentage of patients recover the urinary continence 8 weeks after surgery. With regard to the potency, considered as the ability to achieved and maintain the erection, they reported a significant shorter mean time period in patients with the dHACM application resulting in a early potency return [90].

Another study by Ogaya-Pinies et al. provide encouraging data regarding the enhancement of the functional recovery using dHACM wrap around the neurovascular bundle after radical prostatectomy as an innovation in this clinical field. More specifically, dHACM house and release many important factors involved in tissue repair and growth such as VEGF, TGF-β, FGF, PDGF. Result obtained by follow-up of patients belonging to dHACM group showed a short mean time to return to potency compared with the group of non-graft patients supporting the hypothesis that the application of dHACM is able to accelerate the return to potency. Furthermore, it has been demonstrated that the application of dHACM does not increase the risk of biochemical cancer recurrence (BCR) [91].

4.2 Application of chitosan membrane to improve the functional recovery after nerve sparing radical prostatectomy

In order to improve the regeneration process and the functional recovery of peripheral nerves, different artificial devices have been developed and proposed for clinical application [71,92,93]. Interestingly, the application of membranes made of another biomaterial of natural origin namely chitosan has been reported to be safe and effective on the neurovascular bundles after nerve-sparing robot-assisted radical prostatectomy, appreciating also some positive results regarding potency recovery [94].

Chitosan a derivative of chitin, obtained from the exoskeleton of crustaceans, is achieving resounding interest both in basic research and in clinical settings due to its

biocompatibility, biodegradability, low toxicity and adhesion to the injury site. For this reason, chitosan-based nerve graft have been widely employed for nerve reconstruction as an alternative to autologous nerve graft. The characteristics of chitosan useful in the intra-operative field are (Fig. 4):

(i) Neuroprotective/neuroregenerative effect [84]
(ii) Antitumoral activity [95]
(iii) Antinflammatory and analgesic effect [96]
(iv) Hemostatic activity [97]
(v) Antimicrobial activity [98]

In vitro studies on chitosan membrane showed its suitability as a substrate for proliferation, survival of Schwann cells as well as survival and differentiation of neuronal cells.

The direct contact of Schwann cells with the biomaterial proved its good biological properties allowing important cell functions such as ensheathment, myelination and production of extracellular matrix [78,80,81,85,99].

In addition, several *in vivo* studies showed that chitosan, in form of hollow conduits, achieved promising results improving peripheral nerve regeneration [100-104].

In particular, an *in vivo* study with different degrees of acetylation of chitosan tubes used to reconstruct 10 mm nerve defects in the adult rat displayed functional and morphological nerve regeneration confirmed by stereological analyses. At the same time *in vitro* cytotoxicity was studied to test the biocompatibility of the chitosan tubes showing that the degradation products released by the conduit did not affect negatively the metabolic activity of cells [84,93].

Chitosan nerve conduits have shown promising results not only to bridge somatic nerve defects but also in case of autonomic nerves such as sympathetic and phrenic nerves that following thoracic sympathectomy can be occasionally resected leading to respiratory dysfunction [104].

Recent advancements showed that tissue regeneration can be achieved also by chitosan degradation products called “chitooligosaccharides” (COS) deriving from partially hydrolyzed chitosan with high water-solubility. It is reported that COS are able to support the local microenvironment at the injury site and display neuroprotective effects on neurons making them particularly suitable for medical application [105]. COS support nerve cell adhesion and promote neuronal differentiation and neurite outgrowth through upregulating the expression of both neurofilament and N-cadherin factors [106-108].

Furthermore COS display important interactions with Schwann cells that are essential for nerve regeneration [109].

In addition to its well-known properties useful for tissue repair (biocompatibility, biodegradability, low toxicity), chitosan has also shown excellent potential for supporting three-dimensional organization of regenerating tissues [85,110,111].

Furthermore, another important property of chitosan is represented by its anti-proliferative capability in case of cancer cells.

The mechanism of action of chitosan as an anti-proliferative agent has been well reported, even if the underlying molecular mechanism has not been fully investigated yet. The anti-proliferative properties of chitosan have been tested in several reports with different human cancer cell lines such as breast, gastric carcinoma, melanoma and monocytic leukemia cell lines [95,112-116].

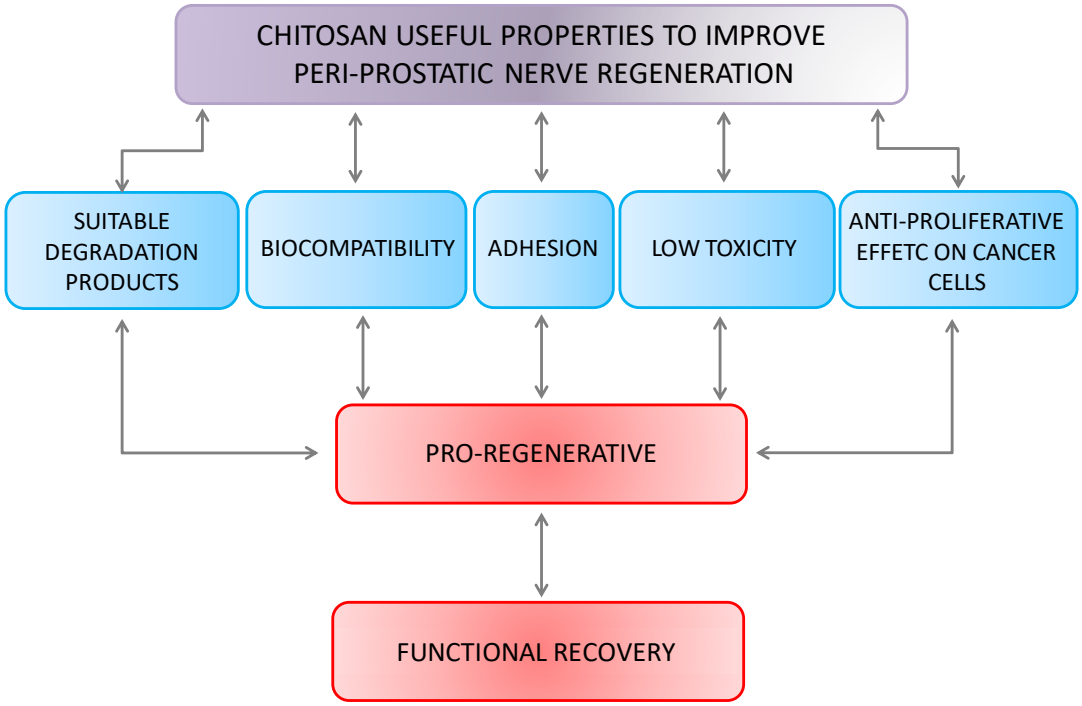

Fig. 4: Schematic representation of chitosan useful properties

DISCUSSION

Erectile dysfunction represents an important impairment in patients following prostate cancer resection, and despite the advancement of the surgical technique such as nerve-sparing robot assisted radical prostatectomy that allowed to preserve the peri-prostatic nerves, the percentage of patients presenting this condition is still considerable.

Furthermore, different pathological conditions such as diabetes can negatively affect the peripheral nerve regeneration, for this reason impairing the functional recovery.

The application of dehydrated human amnion/chorion membranes (dHACM) on the neurovascular bundles has been proposed as scaffold to improve the functional recovery thanks to many growth factors with a neuroprotective, pro-regenerative and anti-inflammatory role. Despite these important properties the application of dHACM has limitations related to the availability and the high clinical cost of the device as well as the controversial use of growth factors in a region in which a tumor has been developed.

The use of a biomaterial such as chitosan, that has already achieved the clinical application for somatic nerve repair, represents a high available and low-cost alternative.

Chitosan has been receiving growing interests among basic and clinical research: results obtained in different *in vitro* and *in vivo* studies showed that chitosan represents an optimal candidate as a neural repair scaffold supporting axonal regeneration.

Its adhesive capability allows the application on neurovascular bundle after robot assisted radical prostatectomy and it can be easily manipulated with the aim to create devices with different structural features.

Moreover, interesting clinical results were obtained by Porpiglia and colleagues reporting a clinical trial in which they tested chitosan membrane, already known for its effectiveness in promoting nerve regeneration [117], as protective device for neurovascular bundles following bilateral nerve-sparing radical prostatectomy [94].

For this reason chitosan could be a suitable scaffold to improve the regeneration of periprostatic nerve and the functional recovery in the context of peri-prostatic nerves regeneration.

Finally multicentre clinical trials will be carried out to study the efficacy of chitosan membrane in the clinical setting.

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