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Review

Nanotechnology for peripheral nerve regeneration

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Abstract

Peripheral nerve injuries (PNI) can lead to lifetime loss of function and disfigurement. Different methods such as conventional allograft procedures and using of biological tubes have problems for damaged peripheral nerves reconstruction. Designed scaffolds with natural and synthetic materials are now widely used in the reconstruction of damaged tissues. Utilization of absorbable and non-absorbable synthetic and natural polymers with unique characteristics can be an appropriate solution to repair damaged nerve tissues. Polymeric nanofibrous scaffolds with properties similar to neural structure can be more effective in the reconstruction process. Better cell adhesion and migration, more guiding of axons and structural features such as porosity provide clearer role of nanofibers for the restoration of neural tissues. In this paper, basic concepts of peripheral nerve injury, types of artificial and natural guides and the methods to improve the performance of tubes like orientation, nanotechnology applications for nerve reconstruction, fiber and nanofibers, electrospinning methods and their application in the peripheral nerve reconstruction have been reviewed.

Keywords: *Peripheral nerve injuries, Nerve reconstruction, Neural guide, Nanofibers*

1. Introduction

Peripheral nerves are the extensive network of nerves that link the brain and spinal cord to all other parts of the body. Peripheral nerves are fragile and easily damaged. A nerve injury can interfere with the communication between the brain and the muscles controlled by the nerve, affecting a person's ability to move certain muscles or feel normal sensations. Several hundred thousands of such traumatic injuries occur each year in Europe and the US alone. The peripheral nerve, when severed is capable of a substantial amount of regeneration. The peripheral nerve contains only the axon part of the neuron and one could consider the peripheral nerve trunk as a protective structure for axons. The cell bodies of sensory neurons are located in the structures just

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next to the spinal cord (dorsal root ganglia (DRG)), or in cranial ganglia, while the cell bodies of the motor neurons are within the CNS (spinal cord or brainstem). Regenerating axons are accurately guided for long distances along naturally occurring Bands of Bungner once the nerve defect is bridged. The most popular approach in peripheral nerve tissue engineering involves *in vivo* implantation of artificial scaffolds and substrates that will guide naturally regenerating axons to the distal segment. Peripheral nerves are discrete trunks filled with sensory and motor axons, and support cells such as Schwann cells and fibroblasts. Due to limb movements and the resulting tensile and compressive stresses, the epineurium provides a protective structure to the axons. The epineurium is a sheath of loose fibro-collagenous tissue that binds individual fascicles into one nerve trunk. Inside these fascicles are the axons, myelinated by Schwann cells [1-3]. Peripheral spinal nerves originate from the dorsal or ventral roots of the spinal cord while cranial nerves originate from the brainstem. Dorsal roots contain sensory axons, carrying signals into the CNS; ventral roots carry motor signals from CNS-originating neurons to muscles and glands (Figure 1). Cranial nerves can be purely sensory or motor, or may contain both types of axons [4,5]. Reconstruction of damaged nerves results from different factors that have been investigated by different methods. Different methods such as utilizing allograft techniques [6], cell therapy such as Schwann cell [7], stem cell, fibroblast and olfactory cells or drug therapy, the use of biological tubes, designed scaffolds with synthetic and natural materials and oriented channels, absorbable and non-absorbable synthetic and natural polymers with unique features, benefiting from new technology of nanotechnology, can improve performance for an appropriate solution to repair damaged nerve tissues that have been reviewed in this article.

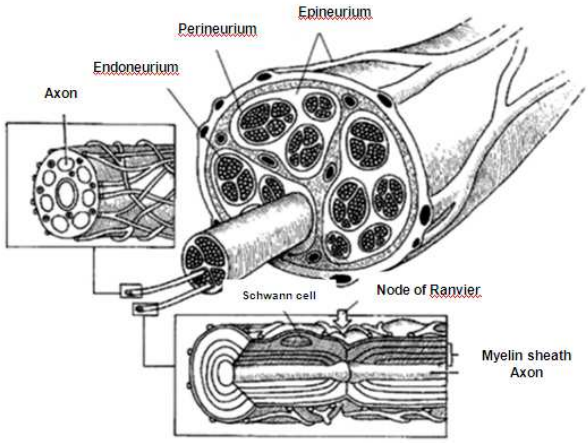

Fig.1. Cross-sectional anatomy of the peripheral nerve. Inset at left shows an unmyelinated fiber

2. Types of nerve guides

2.1. Autologous nerve guides

PNI most commonly results from blunt trauma or from penetrating missiles such as bullets or other objects, but it is also associated with fractures and fracture-dislocations. Crush injuries

are, therefore, more common than nerve transactions. When nerve endings are unable to be rejoined without tension, a bridging section of nerve will be used and two end-to-end sutures are performed. The crushed section of the nerve is cut, removed and replaced by a nerve taken from another (less important) site, typically the sural nerve (from the back of the leg) [8].

The auto graft works relatively well in practice and is the gold standard upon which all alternative therapies are judged. However a second surgery is required to obtain the bridging nerve, and there is loss of function at the donor site, often leading to detrimental changes such as scarring and the possible formation of painful neuromas. Furthermore, donor nerves are often of small caliber and limited in number. These problems drive the search for a tissue engineering alternative to this treatment [8].

2.2. Biological nerve guides

Weiss used non-nerve tissues as alternatives to suture repair of nerve to successfully bridge very short nerve gaps [7-11]. Since then, conduits from many different biological tissues have been used with varying success. These include the use of arteries [10,11], veins [12-14], muscle [15-18], and other materials which are extensively reviewed by Doolabh *et al.* [19]. Other nerve tube conduits have been made from modified biological tissues such as laminin [19] and collagen [20,21] and have proved successful in specific situations. There are a number of disadvantages with the use of blood vessel, muscle and other biologic tissues in bridging peripheral nerve defects including tissue reaction, early fibrosis, scar infiltration and lack of precise control of the conduits' mechanical properties [19]. These limitations have led to the emergence of conduits made from novel synthetic materials, despite potential problems with biocompatibility. Figure 2 shown guides types used for nerve regeneration.

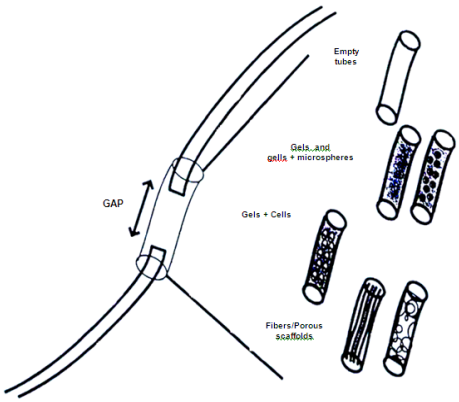

Fig.2. Tubes or guides types for PNS regeneration

2.3. Synthetic nerve guides

2.3.1. Artificial nerve guides made of nonabsorbable artificial materials

Beginning in the early 1980s, replacement surgery, using artificial nerve conduits made of nonabsorbable materials such as silicone (Figure 3), has been in practice for the treatment of

severed nerves, and there are reports documenting partial recoveries with the technique. All these reports however, are of studies demonstrating recovery in morphological continuity of a nerve with a gap as extremely small as about 10 mm in small laboratory animals such as rats, and recovery in motor function has rarely been achieved. The outcome was in no way superior to that of nerve auto grafting in any of the reported studies [22-25].

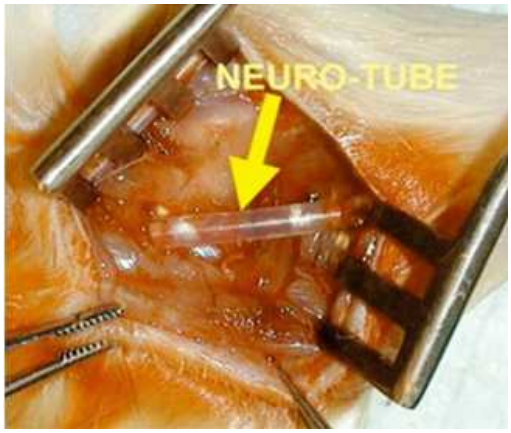

Fig.3. Silicone tube for nerve regeneration

2.3.2. Artificial nerve guides made of absorbable artificial materials

It became recognized from the latter half of the 1980s that degradable-absorbable materials in the body after attaining nerve regeneration are preferable [26]. With the progress in material synthesis and bridging techniques, artificial nerve conduits, made of absorbable synthetic materials, have been developed. Substances such as polyglycolic acid (PGA) and polylactic acid or polyhydroxybutyrate (PHB) [26] (Figure 4) are under investigation as biodegradable-absorbable synthetic materials for nerve regeneration [26].The absorbable and non-absorbable synthetics such as poly lactic acid (PLA), poly glycolic acid (PGA), and their copolymer poly lactic-co-glycolic acid (PLGA) have been widely used for nerve regeneration. All three polymers have been approved by the Food and Drug Administration (FDA) for employment in various devices. These polymers are brittle and they do not have permissible regions for chemical modification; in addition, they degrade by bulk rather than by surface, which is not a smooth and ideal degradation process. In an attempt to overcome the lack of functionalities, free amines have been incorporated into their structures from which peptides can be tethered to control cell attachment and behavior [26]. Khorasani *et al*. designed PLLA tubes for nerve regeneration and studied cellular investigations and in- vitro assessments [27, 29].

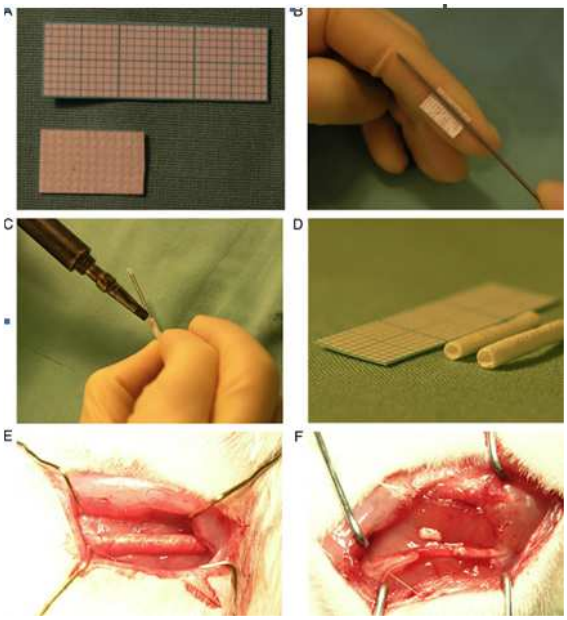

Fig.4. PHB conduit preparations, **A.** PHB material is manufactured as a sheet that can be cut to measure of any size, **B.** PHB sheet is rolled around a 16 gauge needle, **C.** Heat sealing of the rolled conduit, **D.** Rolled and sterilised conduits ready to be implanted, **E.** Implanted PHBconduit at the sciatic nerve site, **F.** Implanted PHB strip at the sciatic nerve site

2.3.3. Artificial nerve guides made of absorbable natural materials

Since the beginning of the 1990s, several reports have been describing that natural biomaterial collagen is satisfactory as a material for regeneration of various tissues/organs and is also useful for peripheral nerve regeneration. Thus, comparative experiments with artificial nerve conduits prepared, comprising collagen extract alone versus autologous nerve grafting, were performed. The results of the study with the conduits were comparable with those of nerve auto grafting, however, and the regenerated nerves were found much inferior to intact nerves. Chitosan and chitin belong to a family of biopolymers composed of a (1-4)-linked N-acetyl-D-glucosamine and D-glucosamine subunits. Figure 5 shows chitosan guides as absorbable natural materials that are used for nerve regeneration.

Prang *et al.* assessed the capacity of alginate gels to promote directed axonal regrowth in the injured mammalian CNS. The multivalent ions used to create the alginate-based gels were copper ions, whose diffusion into the sodium alginate layers created hexagonally structured anisotropic capillary gels. After precipitation, the entire gel was traversed by longitudinally oriented capillaries. The alginate scaffolds promoted adult nerve peripheral survival and highly oriented axon regeneration [30]. This is the first instance of using alginates to produce anisotropic structured capillary gels.

Future studies are needed to investigate the long-term physical stability of the alginate scaffolds, because CNS axon regeneration can take many months long; however, in addition to being able to provide long-term support the scaffolds must also be degradable. Of all the biological and synthetic biopolymers investigated by Prang, only agarose-based gels were able to be compared with the linear regeneration caused by alginate scaffolds. Future studies will also

need to investigate whether the alginate scaffolds allow for reinnervation of the target *in vivo* after a spinal cord injury [30].

Collagen is the major component of the extra cellular matrix and has been widely used in nerve regeneration and repair. Collagen-type I/III scaffolds have demonstrated good biocompatibility and are able to promote Schwann cell proliferation. However, collagen conduits, filled with Schwann cells and used to bridge nerve gaps in rats, have shown surprisingly unsuccessful nerve regeneration compared to nerve auto grafts. This is because biocompatibility is not the only factor necessary for successful nerve regeneration; other parameters such as inner diameter, inner micro topography, porosity, wall thickness, and Schwann cell seeding density will need to be examined in future studies in order to improve the results obtained by these collagen I/III gels [31].

Polysialic acid (PSA) is a relatively new biocompatible and bioresorbable material for artificial nerve conduits. PSA shows stability under cell culture conditions and allows for induced degradation by enzymes. It has also been discovered recently that PSA is involved in steering processes like neuritogenesis, axonal path finding, and neuroblast migration. Animals with PSA genetically knocked out express a lethal phenotype which has unsuccessful path finding; nerves connecting the two brain hemispheres were aberrant or missing. Thus, PSA is vital for proper nervous system development [32].

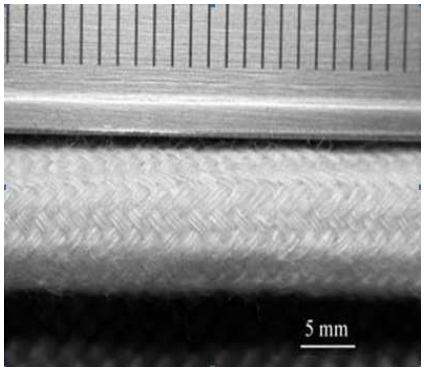

Fig.5. Representative photomicrograph of braided chitosan hollow tubes made from chitsoan yarns through an industrial braiding technique

2.3.4. Composite nerve guides

Perhaps, the best materials for designing a scaffold are both synthetic and natural polymers. Natural polymers such as chitosan, alginate and especially collagen and fibrin due to their structural similarity with the neural structure and properties such as cell attachment have better advantages than synthetic polymers, but the ability to design and swelling, is the problem of the widespread use of such materials. However designs with small nerve gap have been responsive. Utilizing synthetic polymers because of the design control and the use of natural polymers either spongy or fibrous and oriented porous or Hydrogel or coated and other forms can show better function for neural reconstruction. Japanese researchers using polymeric PGA mesh and collagen sponge inside the tube could reconstruct peripheral nerve gap about 3 cm [32,33]. This structure has been shown in Figure 6 [33].

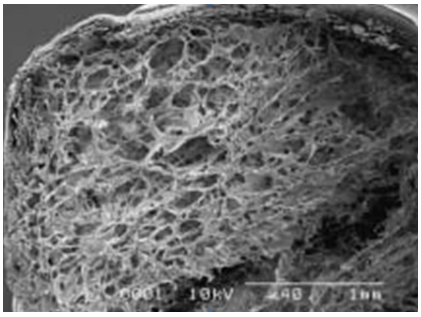

Fig.6. PGA-collagen composite nerve conduit filled with collagen sponge . Electron micrograph (_40)The PGA-collagen composite conduit is filled with a three dimensional sponge matrix

3. Types of neural structures

Super structure is a structure or a cellular scaffold for main tissues simulation. The ways of forming synthetic super structures include the use of hydrogels responding to environmental stimuli such as heat or the form of longitudinal channels ordered or longitudinal fibers and also tensile axons and nanofibrous structures forms [34].

3.1. Longitudinally oriented guides

Longitudinally oriented channels are macroscopic structures that can be added to a conduit in order to give the regenerating axons a well-defined guide for growing straight along the scaffold. In a scaffold with microtubular channel architecture, regenerating axons are able to extend through open longitudinal channels as they would normally extend through endoneurial tubes of peripheral nerves. Additionally, the channels increase the surface area available for cell contact. The channels are usually created by inserting a needle, wire, or second polymer solution within a polymer scaffold; after stabilizing the shape of the main polymer, the needle, wire, or the second polymer is removed in order to form the channels. Typically, multiple channels are created; however, the scaffold can consist of just one large channel, which is simply one hollow tube [34]. Figure 7 shows the preparation method of oriented structures using chemical processes. Oriented structures are created by copper cations. Such oriented structures are used for nerve repair [34].

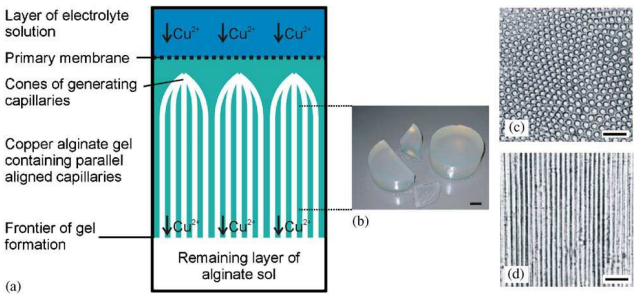

Fig.7. Ultra structure of alginate-based anisotropic capillary gels (ACH), **a.** Illustration of the different phases of anisotropic capillary gel formation, b.Macroscopic appearance of ACH bodies, **c.** ACH in cross- and **d.** longitudinal sections. Scale bars (b) 1 cm; (c) and (d) 100 mm

A molding technique was created by Wang *et al*. for forming a nerve guidance conduit with a multi-channel inner matrix and an outer tube wall from chitosan. In their 2006 study, threaded acupuncture needles through a hollow chitosan tube, where they were held in place by fixing, on either end, the patches were created using CAD. A chitosan solution was then injected into the tube and was solidified, after which the needles were removed, creating longitudinally oriented channels. A representative scaffold was then created for characterization with 21 channels using acupuncture needles of 400 µm in diameter. Upon investigation under a microscope, the channels were found to be approximately circular with slight irregularities all channels were aligned with the inner diameter of the outer tube wall. It was confirmed by micro-CT imaging that the channels went through the entire length of the scaffold. Under water absorption, the inner and outer diameters of the scaffold became larger, but the channel diameters did not vary significantly, which was necessary for maintaining the scaffold shape that guided neurite extension. The inner structure provided an increase in compressive strength compared to a hollow tube alone, which could prevent the collapse of the scaffold onto growing neurites. The Neuro-2a cells were able to grow on the inner matrix of the scaffold, and they were oriented along the channels. Although this method has only been tested on chitosan, it can be tailored to other materials. Lyophilizing and wire-heating process are other methods of creating longitudinally-oriented channels developed by Huang et al. A chitosan and acetic acid solution was frozen around nickel-copper (Ni-Cu) wires in a liquid nitrogen trap; subsequently, the wires were heated and removed. Ni-Cu wires were chosen because they had a high resistance level. Temperature-controlled lyophilizers were used to sublimate the acetic acid. There was no evidence of the channels merging or splitting. After lyophilizing, scaffold dimensions shrank causing channels to be a bit smaller than the wire used. The scaffolds were neutralized to a physiological pH value using a base, which had dramatic effects on the porous structure. Weaker bases kept the porous structure uniform, but stronger base made it uncontrollable. The technique used here can be slightly modified to accommodate other polymers and solvents [35]. Another way to create longitudinally oriented channels is to create a conduit from one polymer with embedded longitudinally oriented fibers from another polymer; then, selectively dissolve the fibers to form longitudinally-oriented channels. Polycaprolactone (PCL) fibers were embedded in a Hydroxyethylmethacrylate (HEMA) scaffold. PCL was chosen over poly lactic acid (PLA) and poly lactic-co-glycolic acid (PLGA), because it was insoluble in HEMA but soluble in acetone. This was important because HEMA was used for the main conduit material and acetone was used to selectively dissolve the polymer fibers. Extruded PCL fibers were inserted into a glass tube and the HEMA solution was injected. The number of channels created was consistent from batch to batch and the variations in fiber diameter could be reduced by creating a more controlled PCL fiber extrusion system. The channels formed were confirmed to be continuous and homogeneous by examination of porosity variations. This process is safe, reproducible and has controllable dimensions. In a similar study conducted by Yu *et al.* HEMA was copolymerized with AEMA to create a P(HEMA-co-AMEA) gel. Polycaprolactone (PCL) fibers were embedded in the gel and then selectively, dissolved by acetone with sonication to create channels. It was found that HEMA in a mixture with 1% AEMA created the strongest gels. When compared to scaffolds without channels, the addition of 82-132 channels could provide an approximately 6-9 fold increase in surface area, which might be advantageous for regeneration studies that depended on contact-mediated cues [36]. Itoh *et al.* developed a scaffold consisting of

a single large longitudinally oriented channel that was created using chitosan tendons from crabs. The tendons were harvested from crabs (Macrocheira kaempferi) and were repeatedly washed with sodium hydroxide solution to remove proteins and to deacetylate the tendon chitin, which subsequently became known as tendon chitosan. A stainless steel bar with a triangular-shaped cross-section (each side 2.1 mm long) was inserted into a hollow tendon chitosan tube of circular-shaped cross-section (diameter:2 mm; length: 15 mm). When comparing the circular-shaped and triangular-shaped tubes, it was found that the triangular tubes had improved mechanical strength, held their shape better, and increased the surface area available. While this is an effective method for creating a single channel, it does not provide as much surface area for cellular growth as the multi-channel scaffolds [37, 38].

3.2. Longitudinally oriented fibers

In addition to longitudinally-oriented channels, longitudinally-oriented fibers can also be added to a conduit to provide regenerating axons with guidance for longitudinally-directed growth. Studies conducted by Newman *et al*. and Cai *et al*. showed that adding filaments to a scaffold promotes inner contact guidance and increases permeability for better nutrient and waste exchange such that the scaffold has superior nerve repair performance over non-permeable conduits that lack filaments [39,40]. Newman *et al.* inserted conductive and non-conductive fibers into a collagen-TERP scaffold (collagen cross-linked with a terpolymer of poly (N-isopropylacrylamide) (PNIPAAm)). The fibers were embedded by tightly wrapping them on a small glass slide and sandwiching a collagen-TERP solution between it and another glass slide; spacers between the glass slides set the gel thickness to 800 µm. The conductive fibers were carbon fiber and Kevlar, and the nonconductive fibers were nylon-6 and tungsten wire. Neurites were extended in all directions in thick bundles on the carbon fiber; however with the other three fibers, neurites were extended in fine web-like conformations. The neurites showed no directional growth on the carbon and Kevlar fibers, but they grew along the nylon-6 fibers and to some extent along the tungsten wire. The tungsten wire and nylon-6 fiber scaffolds had neurites grow into the gel near the fiber-gel interface in addition to growing along the surface. All fiber gels except Kevlar showed a significant increase in neurite extension compared to non-fiber gels. There was no difference in the neurite extension between the non-conductive and the conductive fibers [39]. Cai *et al*. added Poly (L-lactic acid) (PLLA) microfilaments to hollow poly(lactic acid) (PLA) and silicon tubes. The microfiber guidance characteristics were inversely related to the fiber diameter with smaller diameters promoting better longitudinally oriented cell migration and axonal regeneration. The microfibers also promoted myelination during peripheral nerve repair [40].

3.3. Oriented matrices

In vivo experiments with oriented matrices have a higher growth value than isotropic matrices of the same materials. Various cells recognize a three-dimensional geometrical structure on the surface of substrates and their growth can be guided and controlled by fabricating microgrooves on substrate surfaces [41-44]. The cells exhibit sensitivity to the dimensions of the microgrooves [45]. Most of the microgrooves have been fabricated on inorganic substrates, such as micromachined silicon chips [46] which are not very desirable for implantation. Micropatterned regions on glass coverslips with adsorbed laminin have been demonstrated to provide chemical

guidance for axonal outgrowth [47] (Figure 8). The magnetic alignment of positive or negative diamagnetic anisotropic molecules such as fibrin and collagen, respectively, elicits in vitro guided regeneration and improved in vivo regeneration. Also, the methods are for scaffold orientation such as employing magnetic field that has been discussed in section 4.6.

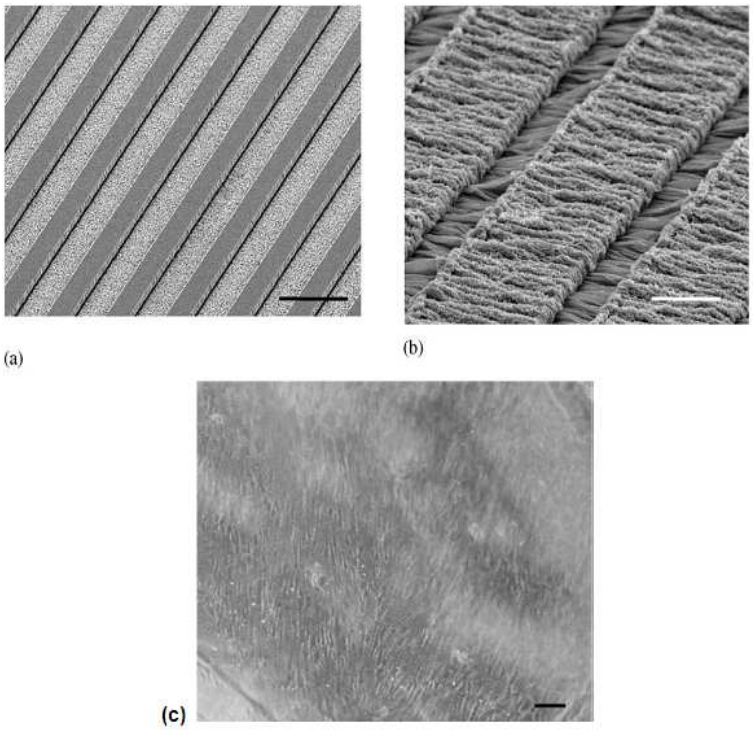

Fig.8. Schwann cells on a smooth compression-molded PDLA substrate Biomaterials, **a,b.** Oriented Schwann cell growth on micropatterned biodegradable polymer substrates **c**

4. Nanotechnology

4.1. Nanometric stretch of axons

Axons in fact are assessed based on their growth experiment with the amount of mechanical tension in the center part of the cylindrical mold. In fact, such a mechanical stretches are created by bioreactor that have four main parts: the expansion chamber designed for axons, linear motion table, motor and controller (Figure 9). The structure of nerve tissue is located within the expansion chamber and change of gases that may be influencing this process is empty that can isolate the cell body. Axons were evaluated in the conditions quite close to the ideal case and vacuum. The Collagen gel is also used for the growth of axons though is not visible with the normal eye. Samples were evaluated by scanning electron microscope or SEM and or TEM that showed signs of growing axons [49]. Also, it was shown that axons could sustain stretch-growth rates reaching 1 cm/day; however, it remained unknown whether the ability to transmit active signals was maintained. It was also shown that stretch-growth did not alter sodium channel activation, inactivation, and recovery or potassium channel activation. In addition, neurons

generated normal action potentials that propagated across stretch-grown axons. Surprisingly, Na and K channel density increased due to the stretch-growth, which may represent a natural response to preserve the fidelity of neuronal signaling (Figure10) [50].

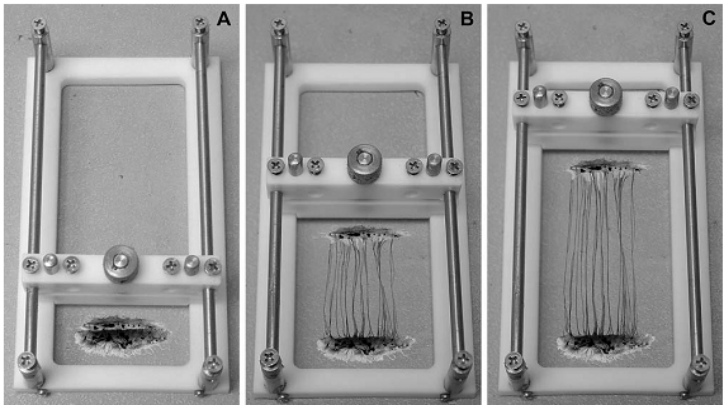

Fig.9. A schematic of axon stretch-growth, **A.** Neurons are plated on two adjoining substrates and are given sufficient time for axons to bridge the two substrates and integrate with neurons on both sides, **B.** The stretching frame displaces one population of neurons away from the other, thereby elongating the interconnecting axons, **C.** Axon stretch-growth is a process that can be gradually induced to achieve a rate of 1 cm/day of growth and to lengths of at least 10 cm

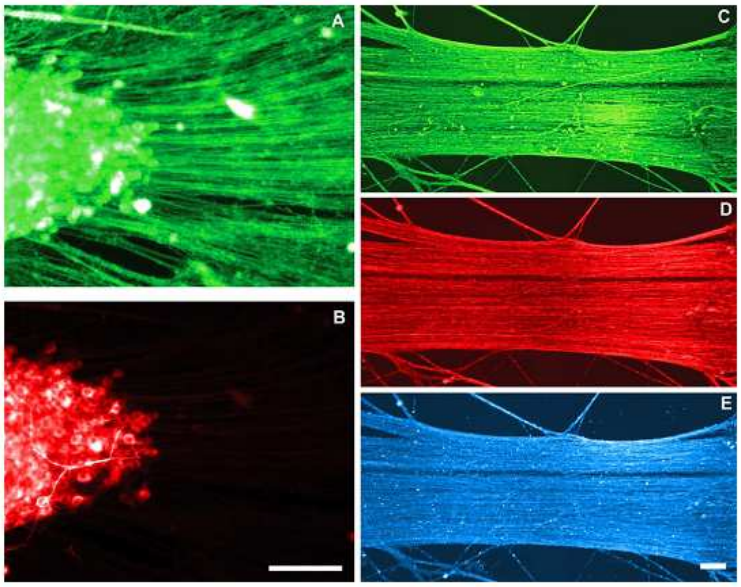

Fig.10. Immunocytochemical analysis of axon stretch-growth, **A.** and **B.** Antibodies against tau and MAP2 were utilized to determine that elongating processes were axons, **A.** The entire length of stretch-growing axons labeled positive for tau protein, **B.** MAP2 was labeled within the cell bodies and was void along elongating processes indicating that these processes are axons, **C-E.** Confocal microscopic images of axons elongated to 5 cm in length. Antibodies against **C.** - tubulin (SMI-61), **D.** 200 kDa phosphorylated neurofilament (SMI-31), and E. tau strongly labeled axons along their entire 5 cm of length. Scale bars, **B.** 50-m, **E**

4.2. Nanofibers

Nanofibers are fibers with diameters one or two orders of magnitude smaller than conventional fibers. These fibers have a uniquely large surface area-to-mass ratio, in the range of 10 m^2/g to 1000 m^2/g (when the diameter is in the order of 500 nm); this makes them suitable for a broad range of applications [51]. Some nanofibers are biocompatible and biodegradable and are used for the replacement of structurally or physiologically deficient tissues and organs in humans. The use of nanofibers in tissue restoration is expected to result in an efficient, compact organ and a rapid recovery process due to the large surface area offered by nanofibers made from protein used for wound healing, the epithelialization of implants and the construction of biocompatible prostheses, cosmetics, face masks, bone substitutes, artificial blood vessels, valves and drug delivery applications [52]. Scaffold materials produced from nanofibers offer a large surface area that can support cell growth. Nanofibers made from silk-like proteins could improve the blood compatibility of implanted prosthetic devices by promoting in vitro and/or in vivo epithelialization of the device, thus, diminishing its thrombogenic and immunogenic properties.

Nanofibers are fibers that are less than 1/1000th the diameter of a human hair. Their applications are wide-ranging in industries including aerospace, filtration, biomedical applications and biotechnology. Nanotechnology has the potential to revolutionize many areas such as surface microscopy, silicon fabrication, biochemistry, molecular biology, physical chemistry and computational engineering as well as raising awareness of nanotechnology in academia, industry and among the general public. The creation of materials and devices at the nanoscale offers unique benefits:

a) increased catalytic efficiency, as a result of high surface-to-volume ratios;
b) increased material strength and hardness, due to fewer physical defects – a corollary to the assembly of nanoscale structures;
c) multiple benefits related to small physical dimensions;
d) faster speeds and improved energy efficiency due to higher packing densities;
e) placement of devices into small structures including biological materials such as cells;
f) novel physical, electrical, chemical, optical, and magnetic properties that are ideal for specific and unique applications.

In the pharmaceutical and medical device industries, nanomaterials and nanodevices may also serve many purposes.

1. Nanoparticles and nanospheres enable the controlled release of therapeutic agents, antibodies, genes, and vaccines into target cells;
2. Biocompatible materials to be used in prosthetics and implantable devices;
3. Fluorescent probes for monitoring biochemical processes;
4. Sensor technologies for the detection and analysis of biologically relevant targets.

Linear aliphatic polyesters such as polyglycolide, polylactide, and their random copolymer poly glycolide-co-lactide are often used as the base materials for implant devices, such as suture fibres and scaffolds for tissue engineering [53-55].These materials meet several controlled-release criteria, i.e. They are biocompatible and biodegradable and they can provide a high efficiency of drug loading. Many different techniques have been developed to produce nanostructured biodegradable materials such as microspheres, foams, and films. It has been demonstrated that the molecular structure and morphology of polyglycolide, polyactide, and their copolymers can play a

major role in the degradation and mechanical properties of the final products [56-62]. The area of research where nanotechnology has had an influence in neural tissue engineering is in developing scaffolds which help in regeneration of damaged nerves. Due to the complex physiology of regenerating nerve, the scaffolds for regeneration require features that aid in the proliferation, differentiation, and migration of neuronal and glial cells. Thus, three-dimensional scaffolds, which mimic the complex physiological properties and chemical cues of the ECM, and which enable the guided cell and axonal migration in-vitro and in-vivo, are necessary.

These scaffolds also need to provide a viable environment for cell growth, not elicit any immune responses, have a high surface area, to allow for the movement of nutrients, and to provide strong support, while being able to degrade at a rate equal to or slower than the rate of regeneration [63]. Nanoscale control over molecular assembly and topography provides the ability to introduce some of the above features with spatial and temporal control, potentially impacting nerve guidance strategies significantly. Three important characteristics of scaffolds that promote nerve regeneration include biocompatibility, degradability, and porosity. In addition, as was discussed earlier, spatiotemporally controlled presentation of topographical and biochemical cues, enabled by nanoscale patterning techniques, can significantly influence regeneration, as described below.

4.3. Topographical cues for regenerating nerves: natural fibers

Natural fibers can be formed from materials such as amphiphilic molecules, silk and collagen. The inherent properties of fiber-based materials make them highly biocompatible, and give them permeability and compliant nature. In particular, permeability allows for the diffusion of needed nutrients that enhance the adhesion and migration of cells. However, these similar properties make the production of such fibers complex; also, the orientation of fiber for guiding cell migration is hard to control [63].

4.4. Self-Assembling Peptide Nanofibers

Natural fibers can be formed through the fabrication of peptide-based amphiphilic molecules. These molecules exist in solution and then self-assemble when introduced to suspensions of cells [64]. Forces from ionic bonding, hydrogen bonding, vanderwaals interactions and hydrophobic responses drive the formation of the peptide self-assembly process and generate a gel-like solid [64]. The hydrophilic heads of the amphiphilic molecules can be designed to include specific epitopes. For example, the incorporation of the sequence isoleucine–lysine–valine–alanine–valine (IKVAV) can help promote neurite growth [65]. Nanofibers with built-in IKVAV are 5–8 nm in diameter and range from hundreds of nanometers to a couple of micrometers in length. When compared to laminin and poly(D-lysine) substrates, cells cultured on IKVAV nanofibers are differentiated more quickly. Additionally, these nanofibers perform better than the coatings of IKVAV soluble peptides, because the nanofibers provide higher density of epitopes [64]. Thus, these fibers with the incorporation of specific epitopes into nanofibers are shown to aid in neuronal differentiation. Peptide-amphiphile nanofibers can also be produced through soft lithography. In this process, a stamp of the fiber orientation is created and then pressed upon a solution of peptide-amphiphilic molecules [66]. This arrangement is then evaporated, sonicated, and dried to produce aligned nanofibers. The dimensions of the fibers

depend on the percent weight of peptide amphiphiles. For the 5 wt% peptide solution, the fibers produced had a width of 200-300 nm with a height of about 55.1 nm while the 1 wt% solution produced a width of 150 nm and a height of 23.1 nm [66]. However, they have only been shown to be compatible with media and further studies need to be done with cell cultures to better determine their effects.

4.5. Silk Fibers to Enhance Nerve Regeneration

Silk fibers represent another class of natural fibers that have been used to promote nerve regeneration. After the removal of harmful materials, which causes adverse immunological responses, the silk was extracted to produce fibers that were 15 nm in diameter. Their effects on DRGs and Schwann cells showed their biocompatibility and ability to promote cell growth [67,68]. Two types of fibers are found including the spider silk fiber and silk worms for tissues reconstruction in tissue engineering, particularly in nerve tissue engineering applications. In addition to adhesion, they are able to support cell migration and are biocompatible and biodegradable; however, their tedious extraction method is a major limitation [69]. Spider silk fibers increase the cell adhesion and proliferation. Jokuszies *et al*. showed that cells are connected fast and quick to silk fibers and grow on silk fiber in a bipolar net with normal speed. They used spider silk fibers for nerve regeneration. For less production of silk from spider has not ever been used in tissue engineering. Of course, it was discovered that species of niphila spider produce a silk that has immunological condition less than to silk of silkworm. The silk nest is a suitable choice from the viewpoint of changeability; of course, its PH is very effective on the basis of arriving to physiology state of PH during the changes. Another advantage of spider silk is its resistance against fungi attack or bacteria for various weeks and it must be noted that they would not also inflate the obtained structure of silk and the strain rate and its changes in this case. But the rate of resistance of the silk on the basis of its composition and different samples and also a separate condition and environmental type used in it will be changed as well.

Attempts have been made for designing different scaffolds and also synthesizing these kinds of fiber. Moreover, the third silk, silkworm is another source of silk. The protein which is obtained from the bombyx silk worm has a core from fibroin with the cover of sericin. Fibroin is a protein with many chains with a repetitive series of hydrophobic and crystal in the face of gly-ala-gly-ala-gly-x which x can be ser or tyr. Sericin has many roots (acid amine) pillars and is more hydrophobic. Silk fibers are so long that thay can be used on the basis of their mechanical resistance and flexibility and of course the rate of their permeability is considered in front of water and oxygen. Moreover, silk fibroin can be easily prepared and sterilized; this silk characteristic is possible to show a series of unsuitable actions. Earlier studies were done in the field of this problem and the result has indicated backgrounds of the effectiveness of sericin. On the basis of this observation it can be said that the silk without sericin has many medical applications. So, the deletion of sericin from around the fibroein is needed before the use of silk. One useful way for its deletion is called degrumming. In this method, the solution of Co_3 - Na_2 which is boiled for deleting sericin has been used without damages to fibroin. Chen young showed that the silk fibroin and the fibroin fibers derived from the solution had a good biological compatibility with cells without cytotoxicity effects [67-70].

4.6. Magnetically Aligned Nanofibers for Cell Alignment

Collagen is commonly used since it is a natural component of the ECM, providing structural strength through fibrous networks. Collagen can also be magnetically treated to produce a gel rod of aligned collagen fibrils. In strong magnetic fields, a high degree of collagen alignment is produced. This was shown through a comparison of collagen gel produced in a magnetic field of 4.7 T and 9.4 T (Figure 11) [70]. As a result, when seeded with DRGs, this alignment facilitated oriented neurite extension. When combined with Schwann cells, directed migration occurred and was further enhanced in the presence of 10% fetal bovine serum [70]. These data suggest that aligned collagen fibers are promising candidates as substrates for guided nerve regeneration.

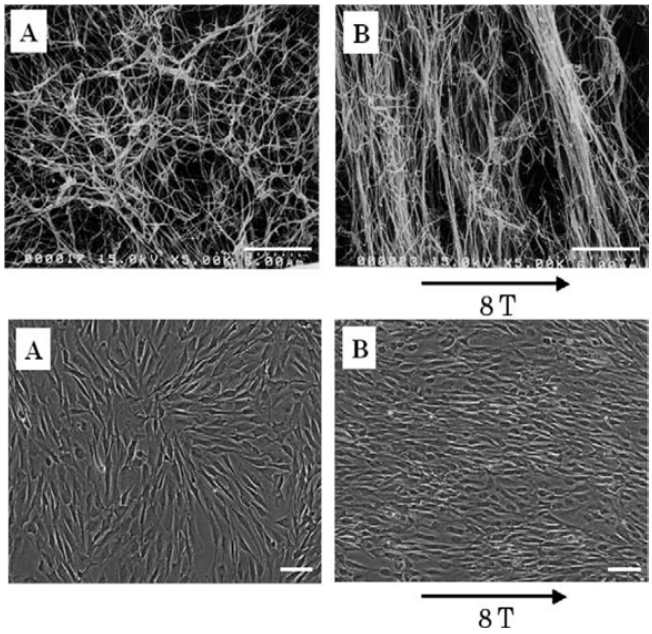

Fig.11. Selected approaches for oriented scaffolds/matrices for peripheral nerve repair. Magnetically aligned structures have so far been demonstrated with collagen . Scanning electron micrographs of collagen with and without 8-T magnetic field exposure for 2 h. The diameter of collagen fibril is about 100nm, **A.** Control group, **B.** Exposed group. Scale bars: 5 mm.Light micrographs of Schwann cells cultured for 60 h with and without 8-T magnetic field exposure, **A.** Control group. ,**B.** Exposed group. Scale bars: 100 mm

4.7. Biosynthetic and synthetic fibers

Biosynthetic and synthetic fibers can be created through a variety of processes including extrusion and electrospinning. While naturally derived polymers including collagen, elastin, and gelatin would be useful starting materials for fabrication of oriented substrates for nerve regeneration, they present technical challenges from a fabrication perspective. One solution may be to mix natural materials with synthetic polymer solutions. To electrospin a fiber, a difference in voltage is used to propel polymer fibers to a target fiber that can have functional groups that can be exploited to aid in cell adhesion [63].

Furthermore, these aligned fibers guide glial alignment and promote directed growth. Like natural fibers, most biosynthetic materials are hard to fabricate due to their inherent properties,

while synthetic fibers are the least problematic to produce and it can be said that electrospinnig is one of the best and novel methods for producing of nanofibrous polymers.

4.8. Electrospinning

Electrospinning is a process by which polymer nanofibers (with diameter lower than 100 nm and lengths reaching meters) can be produced using an electrostatically driven jet of polymer solution. Significant progress has been made in this area over the past few years and this technology has been exploited for a wide range of applications. Most of the recent works on electrospinning has focused either on trying to understand deeper changes to many fundamental aspects of the process in order to gain control of nanofiber morphology, structure, surface functionality and strategies for assembling them or on determining appropriate conditions for electrospinning various polymers and biopolymers. The electrospun nanofibers can even be aligned to construct unique functional nanostructures such as nanotubes and nanowires [72]. Furthermore, depending on the specific polymer being used, a wide range of fabric properties such as strength, weight and porosity, surface functionality, etc. can be achieved. This novel fiber spinning technique also provides the capacity to lace together a variety of polymers, fibers, and particles to produce ultra-thin layers. Small insoluble particles can be added to the polymer solution and encapsulated in the dry nanofibers. Soluble drugs or bacterial agents can be added and electrospun into non-woven mats. Nanofibers provide a connection between the nanoscale world and the macroscale world, since their diameters are in the nanometre range and several meters in length. Therefore, the emphasis of current research is to exploit such properties and to focus on determining appropriate conditions for electrospinning various polymers and biopolymers for eventual applications including: multifunctional membranes, biomedical structural elements (scaffolds used in tissue engineering, wound dressing, drug delivery, artificial organs, vascular grafts), protective shields in specialty fabrics, filter media for submicron particles in the separation industry, composite reinforcement and structures for nano-electronic machines.Figure 12 shows electrospinning apparatus .

Interest in the electrospinning process has increased in recent years. Most of the literature on electrospinning has explored the types of polymer solvent systems from which fibers can be produced. A few studies have also addressed the processing/property relationships in electrospun polymer fibers, either directly or indirectly. Processing parameters of nanofibers have been considered to applied voltage, the solution-flow rate, polymer concentration, molecular weight, and the distance between the syringe needle tip to ground collection plate. Solution viscosity has been found to influence fiber diameter, initiating droplet shape, and the jet trajectory. Increasing solution viscosity has been associated with the production of larger diameter fibers. Baumgarten has also correlated spinning atmosphere with the occurrence of the jet-splaying phenomena. The splaying effects have been observed by Reneker *et al*. Other processing variables, such as acceleration voltage, electrospinning current and distance between the syringe needle tip to ground collection plate have not been investigated totally but have been linked with fiber morphology and defect structures. The background literature for both the electrospray and electrospinning processes suggests that the structure and morphology of the final product is particles or fibers, determined by a synergetic effect of solution parameters and electrostatic forces. These parameters include viscosity, surface tension, concentration and dielectric properties

of the spinning solution and process parameters such as the feed rate of the solution to the syringe needle tip and the acceleration voltage [72,73]. Since natural materials are difficult to spin, they can be co-spun with synthetic polymers. For instance, a conductive polymer, polyaniline (PANi) can be combined with gelatin and then electrospun. Several polymer solutions with varying amounts of PANi were produced and their fibers ranged from 924 to 48 nm in size. The solutions with higher concentrations of PANi produced thinner fibers. Additionally, the proportion of gelatin in the solution should not be less than 5% due to the possibility of beads forming on the fibers. These fibers were then seeded with H9c2 rat cardiac myoblasts, and the cells were shown to proliferate [76].Interestingly, when the width of the fiber was greater than 500 nm, cell alignment was induced. These properties of biocompatibility and conductivity show promise for future implementation in nanofiber scaffolds.

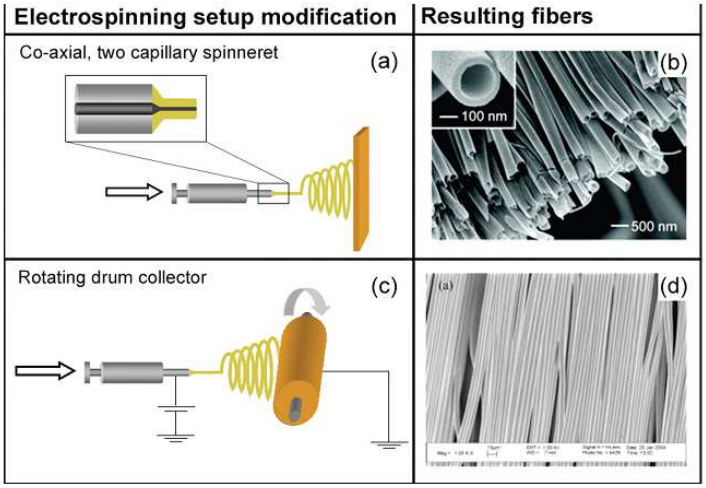

Fig.12. Modifications of the typical electrospinning setup used to produce meshes with unique morphologies, **a.** A co-axial, two capillary spinneret can be used to electrospin hollow nanofibers shown in **b.** A rotating drum collector **c.** can be used to produce aligned fibers **d.** Adapted a. and reproduced **c.** from Li and Xia. Direct fabrication of composite and ceramic hollow nanofibers by electrospinning. Nano. Lett. 4, 933, 2004, **d.** Reproduced with permission from Chew *et al*. Sustained release of roteins from electrospun biodegradable fibers

The most widespread synthetic polymers in neural tissue engineering are poly(a-hydroxy esters) which include poly glycolic acid (PGA), poly lactic acid (PLA), and a copolymer of the two poly lactic-co-glycolic acid (PLGA). These synthetic polymers are frequently used due to their advantageous biodegradable properties and their ease of electrospinning [74,75].Another member, polycaprolactone (PCL), is also used when a slower rate of degradation is desired especially in some drug delivery applications. This method has been used to design neural tubes of different materials like natural polymers of chitosan [78] or synthetic polymers like PLGA [79]. Various factors affect the size of these fibers. This nanofibers increase neural cell adhesion. Figures 13 and 14 shows SEM images of the electrospun PLGA/PCL nerve guide conduit and Longitudinal sections of nerve regenerated within the implanted guide channel. Composite nanofibers [77]. were implied for reconstruction whose results showed that nanofibers had a positive effect .

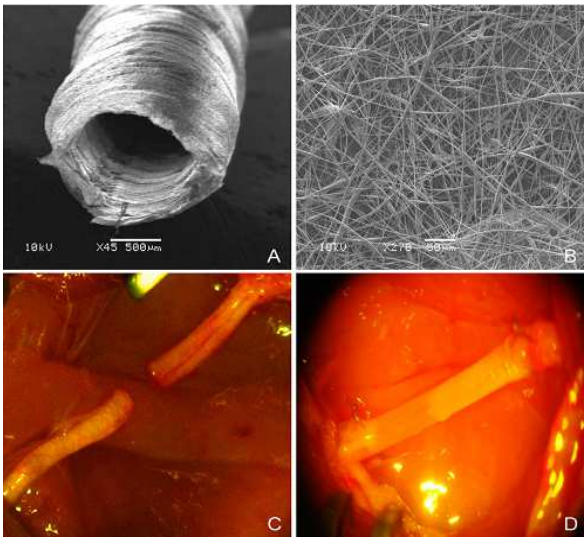

Fig.13. Experimental model. SEM images of the electrospun PLGA/PCL nerve guide conduit **A.** and magnified details of the tube wall **B.** microfibers and nanofibers range in diameter from approximately 280 nm to 8 ìm. The non-woven fibrous microstructure is characterized by small pores (700 nm) and large pores (20 ìm), **C.** Micrograph of sham-operated rat sciatic nerve (experimental group 1), **D.** Micrograph of prosthesis implanted, filled with saline solution and sutured to the transected nerve (experimental group 3)

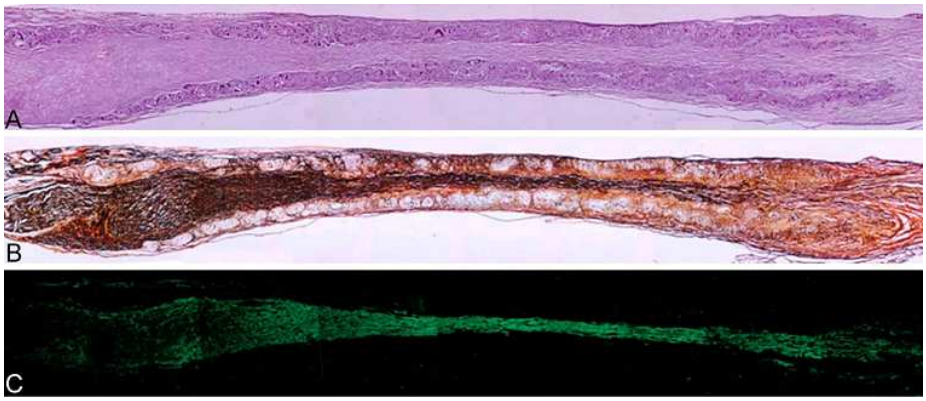

Fig.14. Longitudinal sections of nerve regenerated within the implanted guide channel. In the conduit, the regenerated nerve bridged the 10-mm gap, reconnecting the two sciatic nerve stumps, **A.** 4 months after surgery hematoxylin-eosin staining shows the presence of regenerated tissue filling the conduit lumen; decreased lumen diameter is observable at middle length of the guidance channel. Regenerated tissue positive to Bielschowsky staining **B.** and to anti â-tubulin antibody **C.** shows nervous projections oriented along the major axis of the prosthesis bridging the 10-mm gap between the severed sciatic nerve stumps (image sequence collected at 4× magnification)

5. Conclusion

This article reviewed the various methods of tube designation and the materials used. Utilizing biodegradable synthetic and natural polymers could be good options for nerve regeneration. The designing and engineering hollow tubes or filled with different materials

required methods that achieved factors needed to be like porosity, pores size, and morphology and strength and etc. Nanotechnology was indicated to be able to improve the performance of the scaffold due to the size attribute that played an important role for nerve reconstruction. Employing techniques such as magnetic fields for self assembly and the orientation of nanofibers or nerve cells, or using a controlled design could lead, by the electrospinning method, to good results in nerve reconstruction. Designing tubes composed of composite materials, prepared natural and synthetic polymers with oriented nanofibrous structures, and finally, favorite features could be a good option for designing the scaffold for repairing tissues, especially the nervous tissues that could be the best option for reconstruction.

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