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REVIEW ARTICLE

REVIEW ON CELLULAR SIGNALING, GROWTH FACTORS, AND MECHANICAL STIMULUS IN NERVE REGENERATION

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Abstract

The autonomous and peripheral constitute the entire nervous system. Peripheral nerve injury caused by trauma, accident and other associated factors always results in a huge loss of both the sensory and motor functions. The injured nerves can be successfully restored through the rebuilding of the functional axons. The complete recovery of PNI has not been optimized. Exogenous growth factor (GF) is a new therapeutic strategy that can be used in nerve regeneration. Growth factors mechanism of action is based on the ability to activate the signaling cascades via binding to the individual receptors in order to exert the multiple effects and restore the neuron and tissue regeneration. Although the GFs are limited by their short half-life and rapid deactivation. The use of nerve conduits has been able to reduce these limitations. The nerve conduits have been good biocompatibility and biofunctionality properties.

Keywords: Axons, growth factors, peripheral nerve injury, signaling cascade.

INTRODUCTION

Cellular signaling can be defined changes in cellular homeostasis, which causes cells to respond to different types of stimuli which could be in form of mechanical, electrical and chemical transduction[1]. Cell signaling is a process that enables a cell to interact with itself, other surrounding cells and the host environment[1]. Three major components are involved in cell signaling. They include the: signal, receptor and effector[2]. Signaling could occur in different forms viz the endocrine that involves long range communication, paracrine that involves short range, juxtracrine that involves the contact-dependent signaling and the autocrine. Growth factors are defined as polypeptides that can regulate the differentiation and proliferation of cells[3]. Growth factors that are soluble in nature can easily be incorporated directly into conduits in the nerve region. They play a crucial role in ensuring that numerous cell types that are involved in cell regeneration are supported[4]. Some of the commonly used growth factors include[5]:

- Nerve growth factors (NGFs)
- Glial derived neurotrophic factor (GDNF)
- Vascular-endothelial growth factor (VEGF)
- Neurotrophin 3 (NT-3)
- Leukemia inhibitory factor (LIF)
- Growth associated factor (GAP-43)
- Neurotrophin -4 (NT-4)
- Fibroblast growth factor (FGF)
- Platelet derived growth factor (PDGF)

Nerve growth factor (NGF): It consist of three subunits: γ, β and α. Its main function is in the maintenance of mainly the basal forebrain cholinergic and sympathetic neurons[6]. The main action is based on its ability to bind the tyrosine kinase receptor (trkA) which enhances the choline acetyltransferase expression and its effect on neuron differentiation and maintenance[7]. Nerve growth factors can be increased at the injury site by insertion of Schwann cells into the nerve scaffolds[6]. The neurotrophic factor consists of peptides that are related both in central and peripheral nervous system[7]. Neurotrophins exist as noncovalent homodimers that are biologically active in nature[8,9]. Each molecule of the homodimer is made up of two pairs of antiparallel beta strands. Each of these beta strands is made up of highly flexible short loops[10]. The uniqueness of neurotropins is in their ability to bind to

the receptors which include the tropomysin receptor kinase (TRK) and the tumor necrosis factor (TNF) alpha family of P_{75} receptor. The P45 receptor has similar affinity whenever it binds to neurotrophins, while the tropomysin receptor kinase is more specific in their binding. Nerve growth factors bind to trkA and BDNF, while NT-4/5 subsequently binds to trkB[11].

Neuropoetic cytokines: They belong to the family of pleiotropic glycoprotein molecules that are active in biological activities, induction of immune responses, hematopoiesis regulation, control of cellular differentiation and wound healing induction[12]. The main signal mechanism for neuropoetic cytokine family is carried out through recruiting the common signal transduction receptor subunit[13,14]. Gp130 is not directly activated by neuropoetic cytokines, but they bind to specific ligand-binding subunits. IL-6 binds to the IL-6 receptor, LIF binds to the LIF receptor (LIFR) and the CNTF binds to the CNTF receptor (CNTFR).

Table 1: Relevant studies on chitosan based conduits.

Method of conduit production	*In vitro* analysis	Results	References
Extrusion process, washing and hydrolysis	A short and long term analysis on the 10 mm rat sciatic nerve gap.	No *in vivo* toxicity. Short term: Higher number of activated Schwann cells in the distal segments of nerves.	68
Extrusion process	A 10 mm rat sciatic nerve gap that was repaired for 3 months	No conduit detachment or collapse from the ultrasonography results.	69
Extrusion process, washing and hydrolysis	A short and long term analysis on the 15 mm rat sciatic nerve gap, muscle weight assessment	Higher muscle reinnervation in rats repaired with autograph in comparison with chitosan group. A larger and higher number of myelinated fibers was observed in the autograft in comparison to chitosan experimental group.	70
Freeze-cast process	A 12 weeks repair on a 10 mm sciatic nerve gap with a porous chitosan conduit	Observational of an axonal outgrowth across the conduit	71
Mold-mandrel processing	Characterization of morphological and mechanical properties of chitosan conduit. Repair of 12 mm rat sciatic nerve gap with cell enriched chitosan conduit for 3 months	After 3 months, the conduit became thinner although there was wall and lumen integrity.	72

Brain derived neurotrophic factor (BDNF): Brain and periphery have the highest concentration of this factor. Their major functions are in the promotion of the neuronal and synaptic growth, rapid maintenance of the cortex neurons and the basal forebrain. Its mechanism of action is based on their ability to bind to the trkB receptor and form the BDNF-trkB complex[14].

Growth factors and their role in nerve regeneration

The NGFs belong to the peptide family. Their basic role is to ensure that the nerve fibers differentiate and survive at both the central and peripheral nervous systems[15,16].

Neurotrophins are molecules that are made up of non-covalent homodimer beta chains[17]. They are separated from each other due to the composition of the binding sites. They play a major role in neurotrophic factors because they help the exons in growth cone during regeneration[18].

Glial cell-lined derived neurotrophic factor (GDNF)

They are made up of the GDNF, persephin (PSP), neurturin (NTN) and artemin (ART). The prominent member, GDNF helps in the motor neurons survival, while NTN assists in sympathetic neurons survival[19]. There are two major parts of receptors associated with GDNF. They are the GFRα1 subunit and C-ret subunit. The former serves as the binding site, while the later participates in signaling[20-22].

Interactions between neurotrophic factors

There are differences that exist for both GDNF family and neuropoietic cytokines[23-25]. Damage to the axon leads to significant increase of BDNF mRNA within 8 hrs[26], while in a healthy neuron, BDNF is under expressed, thus within the 7th day of injury, the BDNF level returns to normal. Following external damage, trkB mRNA increases on the second day, while on the 7th day, it reaches the peak. The content and localization of the axonal damage are two major factors that affect the neuropoietic cytokine receptors[27]. After damage to the axon, changes occur at the cellular and molecular level, which are characterized by phagocytic processes[28]. Whenever an injury occurs at the axonal end, the expression of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) increases in the distal part, while the expression of NT-3 and NT-4 neurotrophin reduces[29]. In an intact nerve, the level of NGF mRNA is very low, while in a damaged axon, it increases to 10 times in the distal part within the first 12 hrs. After 72 hrs post-injury, it decreases back to its normal level and remains like that for about three weeks[30-35]. In a damaged axon, the BDNF mRNA increases at the distal part, although the increase is slow when compared to that of NGF mRNA. Although GDNF has been detected in healthy nerve, in a damaged axon, it usually peaks in distal part after 7th day and remains like that for atleast two weeks[36].

Mechanical stimulus (mechanisms, biomaterials, types of stimulus and results)

Ultrasound: Ultrasound can serve two major functions: as a diagnostic and as a therapeutic tool. The mechanical energy generated by the ultrasound helps to

stimulate tissue regeneration[37]. Ultrasound wave can come in either continuous or pulsed. The low intensity pulsed ultrasound is preferable due to the fact that it involves low intensity of mechanical wave in a pulsatile manner, which results in reduction of heat generation[38]. The ultrasound stimulation that regulates intracellular signaling mechanism induction of fibroblasts by mechanical force leads to enhancement of collagen production and also provision of a structural support for axonal repair[38].

Extracorporeal shock wave (ESW)

The difference between extracorporeal shock wave (ESW) and ultrasound is that ESW applies a higher mechanical pressure that is about one thousand (1,000) times compared to that of ultrasound[39]. ESW has a lot of therapeutic applications, among them is in the repair of peripheral nerve injury.

Types of extracorporeal shock wave

i. Focused extracorporeal shock wave (FESW)
ii. Radial extracorporeal shock wave (RESW)

Focused extracorporeal shock wave is applied in deep treatment areas that can reach up to 12 cm, while radial extracorporeal shock wave is applied to a depth of about 3-4 cm[40]. A mechanical stimulus is generated by the extracorporeal shock wave that provokes two major physical effects which include mechanotransduction and cavitation. In peripheral nerve repair, mechanotransduction plays a major role by affecting the development of the gene regulation of the myelin cells, differentiation of Schwann cell and the regeneration of axons[41].

Table 2: Relevant studies on protein based conduits.

Mode of conduit production	Analysis	Results	References
Genepin cross-linked gelatin solution poured into a mandrel	A non-porous and porous genepin cross-linked gelatin conduit were compared and used to repair a 10 mm rat sciatic nerve. Microscopic observation and characterization of the conduit	A faster degradation and lower mechanical strength was recorded in the porous gelatin conduit. There was a significant higher nerve conductive velocity in rats that were repaired with the porous conduit	[79]
Proanthocyanidin cross-linked gelatin solution	*In-vitro* enzymatic degradation and biocompatibility assay. A 10 mm rat sciatic nerve defect was used to repair the proanthocyanidin cross-linked gelatin conduit for 8 weeks	Conduit has resistance to degradation by digestive enzymes. Schwann cell adhesion and growth was supported by gelatin and proanthocyanidin release	[80]
Photo fabrication of the gelatin conduit	10 mm rat sciatic nerve gap was repaired with gelatin conduit for 12 months	At 12 weeks, the gelatin conduit was degraded and absorbed with no signs of any inflammatory reactions	[81]

Peripheral nerve injury repair biomaterials

In tissue engineering, any biomaterial used in nerve conduit production must possess some basic characteristics properties which include: bio-compatibility, biodegradability, permeability, bio-chemical properties, flexibility and resistance to collapse and tension[42]. The biocompatibility property of a biomaterial is further subdivided into 3[43,44].

i). Blood compatibility: This infomr about the ability of the biomaterial not to initiate hemolysis or coagulation in the human body.

ii). Histocompatibility: The surrounding tissues should be free from any side effects.

iii). Mechanical compatibility: The properties presented by the biomaterial must be similar to that of the host tissue. Permeability is another important parameter that should be possessed by a conduit biomaterial. This is because it enhances cell viability and also promotes the exchange of gas, nutrients and waste materials[45]. According to Funakoshi *et al*., a direct relationship exists between the conduit permeability and pore size. To facilitate nerve growth and repair, nerve conduits with large pores are preferable. In nerve regeneration, a semi-permeable conduit is more preferable when compared to both low permeable and impermeable conduits[46]. The nerve guide diameter has a lot of influence on the nerve regeneration outcome. This is because the injured nerve has to match the nerve guide diameter[47]. Conduit wall thickness also has a major role to play in axonal growth. According to Naveilhan *et al*., conduit walls that have more than 0.8 mm thick reduces axonal growth which affects the permeability and porosity reduction which are important factors to consider in nerve regeneration[47]. Another important feature that affects nerve regeneration outcome is the wall thickness[48].

Natural based biomaterials

In nerve regeneration, many natural-based biomaterials have been used. They include polysaccharides such as: hyaluronic acid, alginate, chitin and chitosan. Proteins such as: collagen, gelatin, fibrin and keratin[49].

Polysaccharides

i). Hyaluronic acid (HA): It consist of glycosaminoglycan moiety which is involved in cellular process regulation[50]. Some unique properties associated with hyaluronic acid include: biocompatibility, support of axonal growth and its non-adhesive nature[51]. Although some of the limitations associated with HA which are: fast degradation and low mechanical properties, it can still be used as a conduit internal filler mostly in hydrogel form.

ii). Alginate: Alginate has a wild application in the biomedical field[52]. Chemical reactions is one major way that is used in the modification of alginate. When alginate is oxidized with sodium alginate, it gives rise to alginate dialdehyde[53]. One of the limitations associated with alginate use in promoting nerve

regeneration is its weak mechanical resistance. The physiological loading conditions can be improved when alginate is combined with other polymers[54]. According to Pfister *et al.*, he blended alginate with a biomaterial of natural origin-chitosan which gave rise to a support of nerve regeneration for short nerve gaps. Due to the hydrophilic nature of the chitosan, the blended mixture possessed a good permeability and adequate mechanical strength[55]. The techniques used in the manufacture of alginate include: magnetic templating, electrospinning, gas forming, emulsion freeze drying and 3D printing[56,57]. Alginate can also be used in nerve regeneration as a conduit internal filler that is applied growth factor delivery[58].

iii). Chitin and chitosan: Chitin is a member of the glycosaminoglycan family with the presence of N-acetyl-D-glucosamine moiety. Chitin most abundant in nature is found in the exoskeleton of arthropods[59]. Chitin application spans the food industry, agriculture, pharmaceutics and medicine especially when used in its partial deacetylated form as chitosan[60,61]. There are some unique properties that make chitosan suitable to be used in peripheral nerve regeneration. They include its biocompatibility, ability to support axonal growth and tendency to reducing scar[62]. Although chitosan has low mechanical strength, it can be modified in order to improve its mechanical stability[63]. Other unique properties associated with chitosan include: its versatility and easy modification of the surface structure[64]. An investigation in the nerve regeneration in rat sciatic nerves 3 months after 10 mm nerve repair with chitosan conduits that had three different deacetylation degrees[65]. The study indicated that there was no significant differences among the experimental groups at functional, biomolecular and morphological levels[66]. Reaxon® a chitosan nerve conduit was commercialized in 2015. It was able to bridge nerve gaps up to 26 mm due to some of its unique advantages such as transparency, flexibility and resistance to collapse[67].

Proteins

Collagen: Collagen is used in nerve conduit repair[73]. According to Saltzman *et al.*, a hollow conduit (10 mm) reported better results in rat nerve regeneration and muscle re-innervation than the polyglycolic acid (PGA) filed conduits of collagen. The limitations associated with the use of collagenase in nerve tissue repair is due to its low mechanical stress resistance and weak manipulability[74]. It is recommended that collagen should be combined with chitosan in order to increase its mechanical strength[75].

Gelatin: The thermal denaturation of collagen results in the production of gelatin. Gelatins physical and mechanical properties could be easily altered by using various cross-linking agents[76]. One of the most common cross-linkers used was genipin, a natural substance with low cytotoxicity. According to Chen Y *et al.*, he used a genipin cross-linked gelatin conduit to repair a rat sciatic nerve (10 mm) for 8 weeks. The result obtained after 8 weeks, showed that most of the regenerated axons were not myelinated[76]. Proanthocyanidin was another cross linker that was used to stabilize a gelatin conduit. According to Liu *et al.*, it was used to repair a 10 mm nerve gap and the regeneration after 8 weeks, was assessed. The biocompatibility and degradation rate of the conduit was tested. The *in vivo* studies after 8 weeks showed that the conduit was well integrated into the surrounding tissues[77]. Another natural cross linker used was bis(vinylsulfomethyl). The result obtained after 8 weeks in a 10 mm rat sciatic nerve defect showed that it reduced gelatin swelling and improved its mechanical properties[78].

Silk fibroin

Silk fibroin is used in biomedical applications. It has repeated amino acidic sequence, thus having a very good mechanical property. It can easily degrade[82].

Keratin

It has some unique characteristics that makes it useful as a biomaterial. They include its biocompatibility, biodegradability, bioactivity and its hydrophilic surface. Although it has some limitations such as poor physical and mechanical properties, various cross-linking agents can be used to improve it[86,87]. Gupta and Najak deployed the use of keratin as a protein source for scaffold fabrication. The results obtained showed that they produced a keratin-alginate scaffold[88].

Polyesters

Polyester is a biopolymer that is naturally biodegradable. The type commonly used in tissue engineering is polyhydroxyalkanoates (PHA). Some advantages associated with PHA include pH stability and biocompatibility. One of the limitations of its use is high cost, although it could be reduced to the barest minimum by the development of recombinant microorganisms[88].

CONCLUSION

Overtime, there has been an advancement on the comprehension of peripherous nervous injury, although there is still room for improvement. With growing research on other growth factors, they hold a great promise as a tool for studying intracellular communication among cells.

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AUTHOR'S CONTRIBUTION

Ezegbe CA: investigation, visualization, writing editing. **EA Grace:** review, editing. Both authors checked and approved final version of the manuscript.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICT OF INTEREST

None to declare.

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