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REVIEW ARTICLE

CELLULAR SIGNALING THROUGH THE USE OF GROWTH FACTORS AND MECHANICAL STIMULUS IN NERVE REGENERATION

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Abstract

The nervous system consists of the autonomous and peripheral. Peripheral nerve injury which occurs as a result of trauma, accident and other associated factors always results in a significant loss of sensory and motor functions in an individual. The injured nerves can be successfully restored although it requires a lot of complex cellular and molecular response in order to rebuild the functional axons. When this is achieved, the damaged nerve can accurately connect with their original targets. The complete recovery of PNI has not been optimized. Exogenous growth factors (GFs) are a new and emerging therapeutic strategy that can be used in nerve regeneration. The mechanism of action of growth factor is based on the ability to activate the downstream targets of various 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 associated with short half-life and rapid deactivation in body fluids. The use of nerve conduits has been able to reduce the 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 as perturbations of cellular homeostasis which causes cells to respond to different types of stimuli which could be in form of mechanical (mechanotransduction), electrical (electrotransduction) and chemical (chemotransduction) [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 endocrine (long range communication), paracrine (short range), juxtacrine (contact-dependent signaling) and autocrine. Growth factors are defined as a set of cell-produced proteins and polypeptides which have the ability to regulate cellular proliferation and differentiation [3]. Growth factors that are soluble in nature can easily be incorporated directly into nerve conduits. They play a crucial role in supporting the numerous cell types that are involved in cell regeneration [4]. Examples of growth factors commonly used in nerve regeneration include [5]:

- Nerve growth factors (NGFs)
- Glial cell line-derived neurotrophic factor (GDNF)
- Ciliary neurotrophic factor (CNTF)
- 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): NGF was the first neurotrophic factor to be identified. It consists of three subunits: γ, β and α. Its main function is in the maintenance of basal forebrain cholinergic neurons, sympathetic neurons and nociceptive sensory neurons [6]. The mechanism of action is based on its ability to bind to tyrosine kinase receptor (trkA) which promotes the choline acetyltransferase expression and its effect on neuron differentiation and maintenance [7]. Nerve

growth factors can be increased at the site of injury by insertion of Schwann cells into the nerve scaffolds [6]. The neurotrophic factor consist of structurally and functionally peptides that are related and they mediate potent survival and differentiation effects, 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 two classes of 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 are 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 which play a major role in biological activities, induction of immune and inflammatory responses, regulation of hematopoiesis, control of cellular proliferation/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).

Brain derived neurotrophic factor (BDNF): They are found majorly in the brain and periphery. Their major functions are in the promotion of the neuronal and synaptic growth, maintenance of existing neurons in the cortex and basal forebrain. Its mechanism of action is similar to that of NGF were they bind to the trkB receptor and form the BDNF-trkB complex [14].

The role of growth factors in nerve regeneration

The neurotrophic growth factors belong to the peptide family. Their basic role is to ensure the survival and differentiation of nerve fibers in both the central and peripheral nervous system [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 to guide the exons in growth cone during regeneration [18].

Glial cell-lined derived neurotrophic factor (GDNF)

The GDNF family consist of GDNF, persephin (PSP), neurturin (NTN) and artemin (ART). The prominent member, GDNF helps in the survival of motor neurons, while NTN assists in the survival of sympathetic neurons [19]. They belong to the growth factor-β family of neurotrophic factors. 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].

Ciliary neurotrophic factor (CNTF)

It belongs to the family of interleukin-6. It is during an injury that the production of CNTF increases. The ligand binding of CNTF to the CNTF receptor-α (CNTFR) subunit triggers signals via the Janus kinase-signal transducers and activators of transcription pathway via the formation of a complex with the subunits of glycoprotein-130 [21, 22].

Interactions between neurotrophic factors

There are differences that exist for both GDNF family and neuropoeitic cytokines in terms of receptor systems and related signal transduction pathways [23]. The neurotrophins and GDNF family are homodimeric and biologically active molecules, while neuropoeitic cytokines are long chain α-helix bundle proteins [24, 25]. Damage to the axon leads to significant increase of BDNF mRNA within 8 hours [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 neuropoeitic cytokine receptors [27]. After damage to the axon, cellular and molecular changes occur, and they 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 hours. After 72 hours 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. Ultrasound waves are known to generate mechanical energy which stimulates tissue regeneration [37]. The 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

a. Focused extracorporeal shock wave (FESW)
b. 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]. Extracorporeal shock wave generates a mechanical stimulus 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 myelin gene regulation, Schwann cell differentiation and axonal regeneration [41].

Biomaterials for Peripheral Nerve Injury repair.

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

a. **Blood compatibility**: This talks about the ability of the biomaterial not to initiate hemolysis or coagulation in the human body

b. **Histocompatibility**: The biomaterial should not be able to induce side effects on the surrounding tissues.

c. **Mechanical compatibility**: The mechanical 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*; conduit permeability increases with pore size. Thus 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 proximal and distal stumps of the injured nerve has to match the nerve guide diameter [47]. The conduit wall thickness also has a major role to play in axonal growth. According to Naveilhan *et al*; conduit walls that are 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]. An idea conduit should be easy to suture, and it should be flexible enough to allow the needle to pass via the wall without the escape of the nerve stumps from the conduit lumen [48].

Natural based biomaterials

In nerve regeneration, a lot of natural-based biomaterials has been used. They include polysaccharides such as: hyaluronic acid, alginate, chitin and chitosan. Proteins such as: collagen, gelatin, silk fibroin, fibrin and keratin [49].

Polysaccharides

1. Hyaluronic acid (HA): It is composed of glycosaminoglycan moiety which is involved in regulation of different cellular processes [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.

2. 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, thus it is advisable to use alginate in combination with other polymers in order for it to withstand the physiological loading conditions [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 for growth factor delivery [58].

3. Chitin and chitosan: Chitin is a member of the glycosaminoglycan family with the presence of N-acetyl-D-glucosamine moiety. The most abundant polysaccharide in nature is cellulose, followed by chitin. Its most abundant in nature is found in the exoskeleton of arthropods [59]. Chitin has a wide range of applications in the food industry, agriculture, pharmaceutics and medicine especially when used in its partial deacetylated form as chitosan [60, 61]. 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]. A study investigated nerve regeneration in rat sciatic nerves 3 months after 10 mm nerve repair with chitosan conduits that had three different deacetylation degrees [65]. At the end of the study, 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].

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]

Proteins

Collagen: Collagen is the most abundant protein in the human body, thus one of the main reasons it has been used over the years in nerve conduit repair [73]. According to Saltzman *et al*; 10 mm long hollow conduits reported better results in rat nerve regeneration and muscle re-innervation when compared to collagen polyglycolic acid (PGA) filed conduits. The limitations associated with the use of collagenase in nerve tissue repair is due to its low resistance to mechanical stress and weak manipulability [74]. It is recommended that collagen should be blended with other biomaterials like chitosan in order to increase its mechanical strength [75].

Gelatin: The thermal denaturation of collagen results in the production of gelatin. The mechanical and physical properties of gelatin 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 10 mm rat sciatic nerve 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 was assessed 8 weeks after the repair. 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 bisvinylsulfomethyl. 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 due to some unique characteristics that it possesses. It contains repeated amino acidic sequence, thus having a very good mechanical properties. It is also easily degradable [82]. Mature silk has been shown to possess good tensile and mechanical properties to conduits, when compared to conduits produced with only fibroin solution. The silk fibrin could easily be blended using different biomaterials to reach the target mechanical strength [83].

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]

Fibrin

It is used in scaffold tissue engineering due to its unique properties which include high biocompatibility, versatility, high dissolving and coagulating properties which can be modified [84, 85]. According to Kalbarmathen *et al*; he demonstrated the effect in rat sciatic nerve regeneration of a conduit that was made by fibrin glue to repair 10 mm defects. The result obtained indicated that the fibrin glue demonstrated a better axon regeneration length in comparison PHB conduits 2 weeks after the repair [85].

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, it can be improved by using various cross-linking agents [86]. When keratin is used as a hydrogel-filler for conduits in mice, it has proven to be effective in promoting nerve regeneration in short gaps of 5-15 mm [87]. Gupta and Najak used keratin as a protein source for scaffold fabrication. The results obtained showed that they produced a keratin-alginate scaffold [88].

Polyesters

A polyester is a biopolymer that is naturally biodegradable. The most commonly used type 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 [89].

CONCLUSIONS

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. **Okafor N:** writing, editing. **Ezegbe AG:** supervision, review, editing. **Juliana Marchi:** supervision. **Anikwe Celestine:** writing, editing, review. **Okorafor Ezinne:** writing, review, editing. All 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

Authors declare no conflict of interest

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