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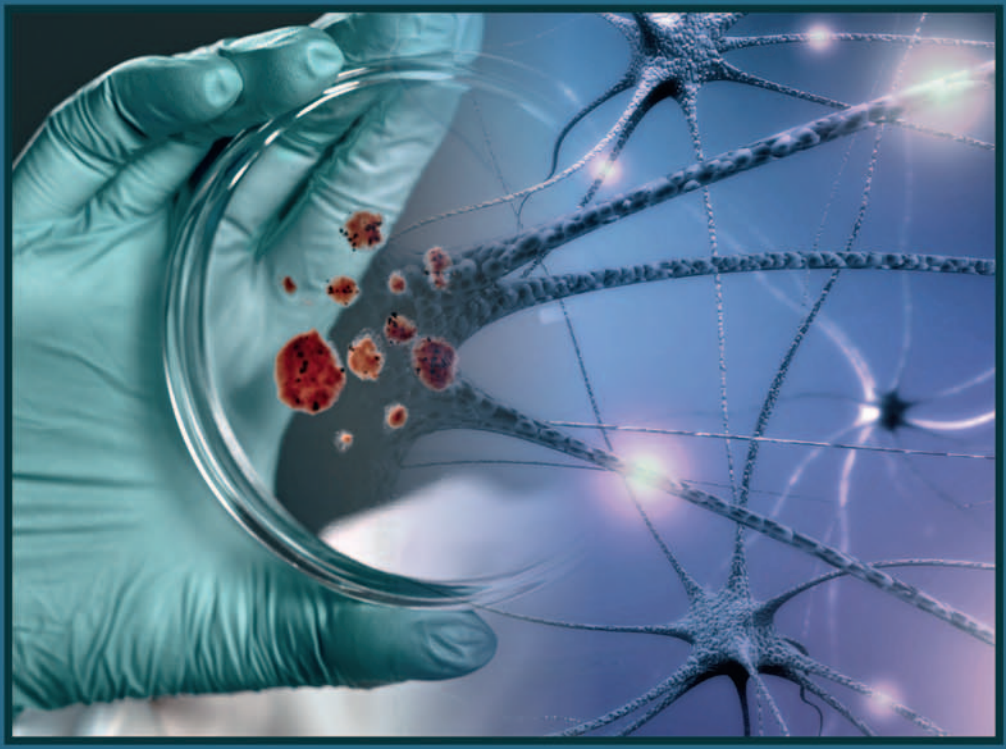

BIOMATERIALS FOR NEURAL TISSUE ENGINEERING

Edited by
OGUZHAN GUNDUZ
CEM BULENT USTUNDAG
MUSTAFA ŞENGÖR

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CHAPTER 7

Growth factor delivery for neural tissue engineering

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7.1 Introduction

The Nervous system (NS) is the complex well-organized cell collection in charge of controlling, coordinating, communicating, and directing all essential functions of our internal organs/body. Anatomically, it consists of two main components: the central nervous system (CNS) and the peripheral nervous system (PNS). Functionally, it is subdivided into the somatic nervous system and the autonomic nervous system (visceral) [1]. The CNS is the brain and spinal cord, while the PNS comprises all nerves except the CNS [2]. Disease, trauma, and disorders cause injuries to both the PNS and CNS [3]. However, PNS injuries, which place a great burden on individuals and health systems, are seen in more than 100,000 people each year in the USA/Europe, and are caused by different traumas [4]. Although PNS injuries are not life-threatening, they may result in a lifetime loss of function and disfigurement [5,6]. The injuries of CNS caused by physical damage, neurodevelopmental disorders, and chronic neurodegenerative diseases damage brain architecture, resulting in loss of neuronal cell bodies, axons, and glial support [7,8]. Normally, the NS begins to self-regenerate and repair itself almost immediately after an injury. However, neural regeneration in the CNS is restricted due to CNS axons' limited capability of regeneration in contrast to the regeneration ability in the PNS [9]. Axons in the PNS readily regenerate after an injury [10]. However, in some cases (e.g., large peripheral nerve injuries (PNI) > 1 cm), the regenerative capacity of the PNS becomes insufficient without any additional surgical/therapeutic intervention [11]. Despite the understanding of the biological mechanism of the NS regeneration, repair mechanisms of the nervous tissues still remain a challenging issue [12]. The complex structure and

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function of the NS make limited regeneration and treatment of neural tissues become more difficult in comparison to other human body tissues. Current therapeutic approaches are unable to fully restore nervous system injuries, and there is still a lack of optimal treatment for perfect and complete functional recovery [13]. Therefore, it's crucial to develop a biomimetic strategy that can provide optimal morphological, chemical, and biological signals for nerve tissue recovery [1]. Therefore, nanotechnology and nerve tissue engineering (NTE) provide new alternative therapeutic approaches for the effective manner of nerve tissue repair [14].

Regenerative medicine including tissue engineering (TE) is an innovative strategy used to treat patients with organ/tissue loss or organ/tissue failure in order to repair/regenerate dysfunctional tissues [15]. This field, which combines knowledge of cell biology, material science and engineering, chemistry, and nanotechnology, is constantly growing in order to restore the biological and functional characteristics of damaged or ill tissue [16,17]. NTE and regenerative medicine are research fields that combine engineering and neuroscience principles to make it easier to regenerate or repair neural tissues that have been harmed by ischemic, thermal, chemical, mechanical, pathological, or aging factors [18]. The main focus of NTE is the development of biomimetic environments or external biomaterial supports consisting of cells and bioactive molecules to repair and restore neural tissue function [1,19]. Successful physical support for nerve regeneration with 3D scaffolds results in better host tissue engraftment following new tissue growth to facilitate cell function. An ideal scaffold for NTE should meet the following criteria: (i) biocompatibility; (ii) biodegradability; (iii) neural transmission relies on the action potential generated at the synapse [19].

Growth factors (GFs) are soluble proteins, and a wide variety of complex bio-inspired systems are being developed to maximize their effectiveness. Today, it is aimed to improve tissue repair and cellular regeneration by controlling how much, when, and where GFs are released [20].

Nanoparticles, nanofibers, hydrogels, microneedles, and graphene-based materials are mostly used for GF delivery into desired areas. In this chapter, growth factor delivery for NTE is examined.

7.1.1 General GF

GFs or neurotrophic factors are soluble proteins that are naturally secreted from cells and play an important role in the regulation of various cellular

processes (i.e., proliferation, migration, and differentiation, by binding to specific transmembrane receptors on target cells) [20–22]. The chemical structure, concentration, sequence, and application of multiple GFs can have a significant impact on the healing of the damaged area [20]. Although technical advances, such as delayed or cell-specific genetic manipulations, offer many opportunities to study later stages of neural development, cross-talk and redundancy between growth factor signaling pathways remain formidable barriers [20].

Glial cell–derived neurotrophic factor (GDNF), Nerve Growth Factor (NGF), Neurotrophin-3 (NT-3), and Vascular Endothelial growth factor (VEGF) are the ones most commonly used in combination or individually [23].

7.1.2 Neural GFs and roles in NTE

Nerve growth factor (NGF) has a crucial role (Fig. 7.1) in maintaining the function of cholinergic neurons in the forebrain basal (medial septum, Meynert's nucleus basalis, and Broca's diagonal band) [25]. Playing an important role in the neural development of the central nervous system (CNS), NGF is the first discovered member of the neurotrophin family and is involved in numerous physiological functions in adults, such as endogenous secretion, phenotypic and functional maintenance of sensory neurons and sympathetic fibers in the peripheral nervous system [26]. As a result of the studies, the researchers found that the addition of NGF to the nanofiber scaffolds increased neurite outgrowth and performed better in nerve regeneration [27].

Heparin binds to growth factors such as fibroblast growth factors, hepatocyte growth factor (HGF), platelet-derived growth factor, vascular endothelial growth factor (VEGF), and bone morphogenic protein-6 with high affinity (BMP-6). Heparin binds to these growth factors and keeps them stable by preventing thermal degradation. Fibroblast growth factor-2 (FGF-2) promotes self-renewal of many types of stem cells, including neural stem cells, keeps stem cells in a primitive state, increases proliferation of endogenous neural precursors in the subventricular region following traumatic brain injury, and promotes neural stem cell differentiation. It also increases neuronal migration and differentiation [28].

Mattson and Rychlik investigated the effect of glia on "natural" and excitatory amino acid (EAA)-induced neuron death in embryonic rat hippocampus cell cultures containing astrocyte-like glia and neurons. As a

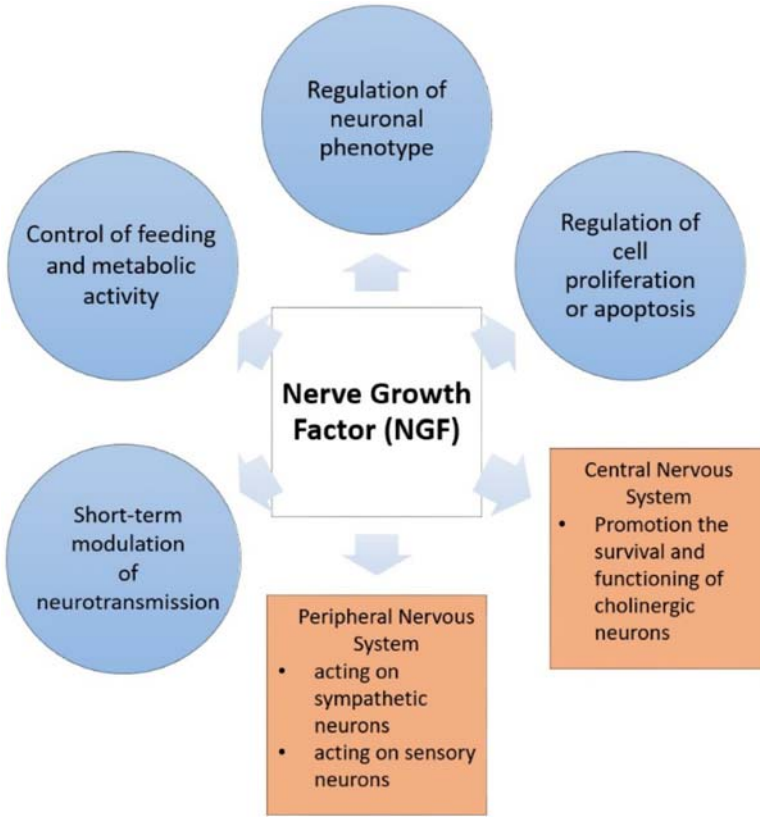

Figure 7.1 The cholinergic system, nerve growth factor, and the cytoskeleton [24].

result, they discovered that neurons that came into contact with glia survived longer than neurons that did not. Glia was found to have FGF-like immunoreactivity. The addition of FGF to cultures of an antiserum produced against an internal peptide fragment significantly reduced glial protection against both spontaneous and EAA-induced neurotoxicity. Exogenous FGF or contact with glia also protected hippocampal neurons from Ca^{2+} ionophore-induced degeneration, implying that FGF improves neurons' ability to handle a Ca^{2+} load [29].

Angiogenesis and functional recovery can be aided by Vascular Endothelial Growth Factor (VEGF). However, needle-induced injury, inhomogeneous VEGF distribution, and limited VEGF retention in the brain after intracranial or intravenous injection limit its use [30].

7.2 Biological considerations

In drug delivery systems (DDS), material selection is an important criterion. Especially, the materials must not cause unfavorable cellular and tissue reactions. In some cases, biodegradability is also an important aspect; therefore, degradation should ideally occur while new tissue is forming. Another consideration is that selected material mimics the extracellular matrix (ECM) and physical characteristics of the tissue. Thus, materials can be easily manipulated with other materials such as hydrophobic polymers and composites. Finally, the size and structure of GFs or other types of drugs are important considerations in determining the interaction with the materials in vivo. In particular, the molecular weight of GF plays a crucial role in the interaction with the extracellular space. Therefore, these considerations should be considered when developing delivery systems for growth factors in NTE applications [31].

7.3 Material selection

The selection of appropriate scaffold material to promote neural and nonneural cell differentiation is important for NTE (Fig. 7.2) [18]. Polymers are materials which have been largely used for designing a suitable scaffold in NTE [33]. They regulate the biological processes that control axonal growth, facilitate tissue repair, and promote integration into already-

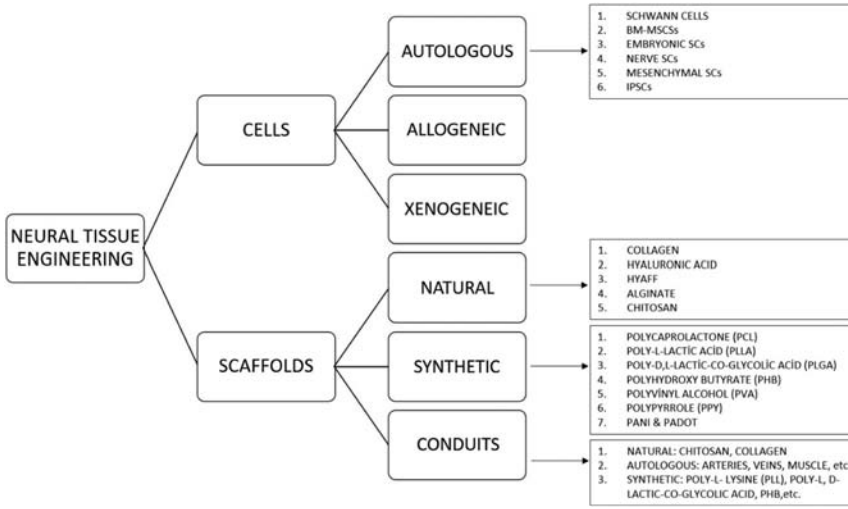

Figure 7.2 Materials used in NTE [32].

existing healthy tissue. Additionally, they support growing neurites mechanically and prevent the formation of scar tissue [34]. In NT applications, polymers with various physical, chemical, mechanical, and inherent biological features are widely used as scaffolds, hydrogels, nerve channels, and drug delivery systems. The two types of polymers used in damaged nerve tissues are natural and synthetic (Fig. 7.3) [16].

- Natural Polymers

Natural polymers have a long history in tissue engineering (TE) due to their high biocompatibility and natural biodegradation kinetics. Natural polymers are analogs to macromolecular substances existing in the human body and have the same or very similar structure to the injured tissue. They minimize the risks of cytotoxicity and an immune reaction after being implanted in the body because they do not interact with the biological systems of the host. In NTE, natural polymers take various functions, such as gelling agents, matrix formers, or drug release modifiers. Also, they have excellent properties for cell adhesion and growth. However, the weak mechanical properties, thermal sensitivity, and processing difficulties of natural polymers limit their use alone. For this reason, they are generally used with synthetic or electroconductive polymers. Natural polymers applied in NTE are originated either in animals or plants [1,16]. The most

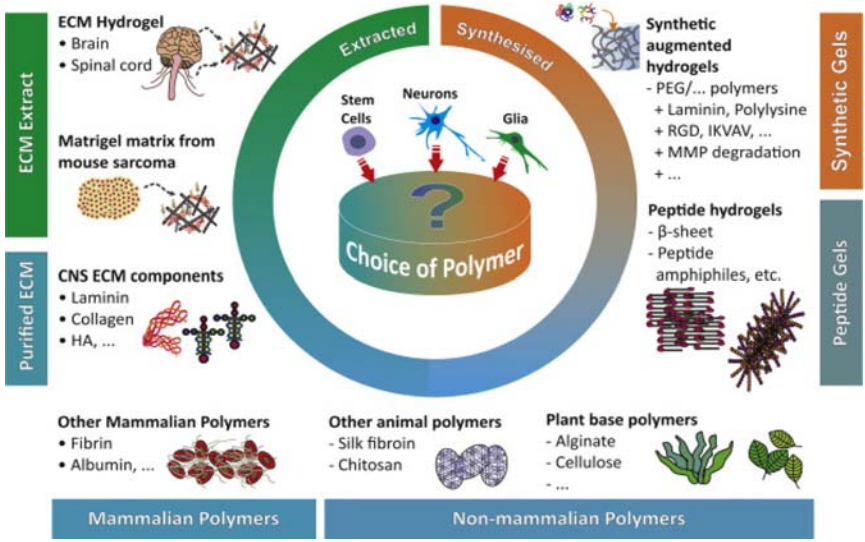

Figure 7.3 Material selection of NTE applications [35].

researched type of natural polymers in NTE are collagen, gelatin, elastin, alginate, hyaluronic acid, chitosan, keratin, and silk [32].

Collagen is the most abundant protein in connective tissue, providing structural support and assisting multiple functions in the body. There are 28 known types of collagen, with type I being the most common that can be implanted inside the body due to the fact that many people have an innate immunity against it. Low antigenicity, low inflammatory and cytotoxic response, high biocompatibility and mechanical strength, and cross-linking ability are the most important advantages of collagen. Although collagen offers many possibilities for NTE, it lacks mechanical strength and structural stability. It can be physically or chemically cross-linked to increase mechanical strength. In another way, it can be used by combining with other materials such as natural, synthetic polymers, and inorganic materials [36].

Collagen is a popular biomaterial for use as nerve guide conduits (NGC) for many years [37,38]. According to studies, collagen-based NGC has been used to completely repair small nerve injuries (5 mm gap) [39] and partially reconstruct larger nerve injuries (15 mm in rat and 35-mm gap in dog sciatic nerve model) [40,41]. Collagen is also very important in NTE applications because it is the only polymer that has approval for clinical tests [16]. Several commercial collagen-based nerve conduits such as NeuroMatrix, Neuroflex, and NeuraGen have been approved by the FDA [37].

Gelatin is a natural biopolymer obtained from the acid or alkaline-based hydrolysis of collagen under controlled conditions. Gelatin's unique properties of high biocompatibility, biodegradability, and low cost make it a good candidate for application in the pharmaceutical, food industry, and cosmetics. In addition, gelatin is less allergenic and toxic than collagen [16]. The chemically modifiable nature and structure of gelatin enhances the cell adhesion, proliferation, and integration in TE [42]. Gelatin is generally used together with synthetic polymers due to its weak mechanical strength and rapid degradation rate in the physiological medium. While the mechanical properties of gelatin increase with its combination with other materials, it increases the cell attachment, migration, proliferation, and differentiation of the materials to which they are added [43,44].

Gelatin was firstly used as an electrospun form in NTE applications by combining with synthetic and natural polymers. The electrospinning method for fabrication technique offers advantages because of controlling and optimization of mechanical, biological, and kinetic properties [16]. According to studies, gelatin combined with PCL nanofibers had a supporting role in the neurite outgrowth and Schwann cells proliferation

in vitro and in vivo [45–48]. In addition to nanofiber forms, gelatin-based nanoparticles [49] and three dimensionally (3D) printable bioink form [50] are used in NTE applications.

Elastin is a popular protein in TE applications due to some of their excellent properties. It is well characterized by prolonged stability, slow degradability, elasticity, self-assembly, and bioactivity properties. Elastin is one of the polymeric ECM protein which is essential for elasticity of organs and tissues. Although elastin is important in soft tissue regeneration, it is not widely used in NTE applications. However, elastin-like polypeptides (ELP) inspired by human tropoelastin have recently gained importance due to their ease of use in NTE applications because of their biological and physical properties. The most significant advantages of ELP are that their molecular weights and sequences can be easily controlled and their physicochemical properties can be modified by cross-linking or combining with other polymers [51]. In addition, ELPs are thermally responsive polymers that can dissolve below the transition temperature and aggregate above the transition temperature. Therefore, they present an appropriate medium for controlled DDS in the treatment of neurodegenerative disorders [52–55].

Alginate is one of the linear, homogeneous, and anionic polysaccharides widely distributed in the cell walls of brown algae. Its high biocompatibility and hydrophilicity, low toxicity, cost-effectiveness, and high gelation features make it a particularly interesting material for TE applications. However, a major drawback of alginate is the presence of impurities which are attributable to its marine origin. The organic and inorganic-based various impurities such as proteins, endotoxins, polyphenols, and heavy metals could be present in its nature. Therefore, a multistep extraction procedure must be applied to obtain high purity alginate [56,57].

Alginate-based materials can offer appropriate properties as scaffolds for neural tissue regeneration. However, the short-term mechanical strength and rapid degradation are the most common difficulties of alginate. For this reason, producing and applying hybrid scaffolds by combining alginate with other polymers to improve mechanical properties of alginate will also give good results in NTE applications [58].

Hyaluronic acid (HA) also known as hyaluronan (HA) is a long, unbranched polysaccharide molecule which is found in all tissues in the body, including brain extracellular matrix [59]. HA has been investigated for TE applications because of its modifiable properties like biocompatibility, biodegradability, and hydrogel forming ability [60]. There is a significant role in maintaining homeostasis in neuronal tissue through affecting cell

behaviors such as cell proliferation, migration, and differentiation [61]. HA has great potential for PNS regeneration and therapeutic potential for the CNS due to its existence in the brain ECM. In PNS applications, HA supports 2D or 3D neurite outgrowth, potentiates proliferation of neural precursor cells. The advantage of using HA comes from its excellent biocompatibility, degradation to safe products, low immunogenicity, and specific viscoelastic properties for different tissue types. In addition, it is able to bind to specific cell receptors [32]. One problem with the application of HA is related to its solubility in water. However, this can be handled by adding additional components to HA and applying cross-linking processes.

Chitosan is one of the natural polysaccharide polymer which is obtained from chitin by the deacetylation process. It is similar to the glycosaminoglycan, one of the components of the native (ECM) that provides an ideal microenvironment for cell adhesion and proliferation due to polar groups in its structure [62]. Chitosan possesses numerous superior features such as gel forming ability, biodegradability, excellent biocompatibility, and cost-effectiveness. In addition, it is noncytotoxic and its antibacterial activity is quite high. Besides, chitosan is a versatile polymer that can be used in the form of membranes, sponges, gels, beads, and tissue scaffolds [57,63,64].

Chitosan and its derivatives have been demonstrated to enhance axonal regeneration and anti-inflammation. It successfully delivers neurotrophic factors in NTE and regenerative medicine [65]. According to studies, chitosan can promote the proliferation and differentiation of nerve cells in the direction of the neural network [66,67]. However, chitosan must be combined with other synthetic polymers to improve its performance in NTE applications because of its rapid degradation rate, limited flexibility and elasticity, and high hydrophilic surface properties [68].

Keratin is a protein that is made up of various amino acids linked together by the disulfide cysteine amino acid and is primarily found in hair, nails, skin, wool, hooves, feathers, and horns [34]. Since keratin is similar to the macromolecular substances or extracellular matrix (ECM) in the body, it has great advantages in terms of biocompatibility, biodegradability, and low immunogenicity. Also, keratin provides high cell attachment and proliferation due to its adhesion sequences and is modifiable to desired particular tissue. In recent years, keratin protein has demonstrated that is a good candidate material for biomedical applications due to its self-assemble property. In the solution, keratin self-assembles to fibrous structures which have ability to promote cell adhesion for peripheral nerve regeneration [52].

Among natural biomaterials and their applications, keratin is one of the first candidate materials with high potential for NTE due to its biological properties. Numerous studies showed that keratin-based materials are neuroinductive and capable of triggering tissue regeneration in peripheral nerve damages. It influences attachment, proliferation, and increased the activity of Schwann cells. Keratin-based hydrogels are able to offer biocompatible structures which can help axonal outgrowth and neural cell adhesion due to its excellent property of biodegradability [52].

Recent studies have shown that keratins are produced as nanoparticles, nanofibers, and hydrogel forms by combining them with different polymers for different purposes. For instance, the hydrogel based on the combination of keratin and PCL is able to repair critical nerve injury by promoting axon migration and gene expression of Schwann cells [69]. In another example, electrospun PVA/keratin nanofibrous scaffold allowed for significantly facilitating morphology, adhesion, and proliferation in vitro [70]. However, less mechanical strength of keratin is the main drawback in TE and NTE applications. Also, the extraction of keratin is more difficult and solubility is less than in other polymers.

Silk is a natural fiber of fibroin protein produced by numerous species including spiders and silkworms. Especially, silkworm silk is the most known type in biomedical applications due to its excellent mechanical properties, high biocompatibility, low toxicity, limited bacterial adhesion, biodegradability, and ease of processing properties [71,72].

Silk is a versatile material that can be used in hydrogels, tissue scaffolds, nanofibers, and nanoparticles, among other applications. Particularly, silk-based hydrogels are often used in NTE because of their ability to protect structural integrity. According to studies silk hydrogels have been used as scaffold for helping of neurons' differentiation in the regeneration of brain and nerve tissue [73]. Furthermore, silk fibroin films showed biocompatibility, slow degradation, and nontoxic property in vitro. Therefore, it can be a good candidate for long-term applied nerve guide in the treatment of CNS injuries [74].

- Synthetic Polymers

Synthetic polymers for NTE applications include a large subfamily of materials. They are divided into biodegradable groups that are useful in tissue engineering applications due to their tailored properties or nonbiodegradable groups that are resistant to biodegradation in biological mediums. The lactic and glycolic acid—based polyesters and their copolymer poly(lactic-co-glycolic acid), and polyethylene glycol, PEG-based

hydrogels are considered biodegradable; while biomaterials including methacrylate are commonly nonbiodegradable [34]. Initially, the same materials used for skin grafting and surgical repairs of peripheral nerves were employed to fabricate neural scaffolds. However, new scaffolds have been produced due to developments in biomaterials chemistry, production technologies, and sectors of manufacturing [34]. Because of their various characteristics, including mechanical strength and flexibility, biodegradability, nontoxicity, noninflammatory response, and porosity properties, synthetic polymer-based scaffolds have been used for both PNS and CNS injuries, both in vivo and in vitro. They can be modified to fabricate a wide range of mechanical features and degradation rates. However, hydrophilicity is the main critical issue for synthetic polymers. In contrast to hydrophilic materials, hydrophobic materials tend to encourage monocyte adhesion, which leads to a local immune response at the implant site. This feature can also be easily addressed by modifying the synthetic polymers [75]. The most widely used synthetic polymers for NTE are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymer poly(lactic-co-glycolic acid) (PLGA), which are thermoplastic-based polymers. In addition, polyethylene glycol (PEG) and poly (2-hydroxyethyl methacrylate) (pHEMA) are hydrophilic-based synthetic polymers that are commonly used for NTE applications.

PLA is a linear aliphatic polymer used widely as a biomaterial in different clinical applications [33,76]. It has excellent properties compared to other aliphatic polyesters such as high mechanical strength, controllable degradation rates in vivo, low toxicity, bioabsorbability, biodegradability, transparency, and easy processability [77,78]. The major advantage of PLA is biodegradability. Because of its unique properties, PLA has wide biomedical application areas. In particular, it can be considered as an ideal cell environment in NTE. Generally, the development of PLA-based neural scaffolds is for supporting Schwann cells, axon extension, and vascular growth [79]. According to studies, neural stem cells (NSCs) can develop on aligned poly (L-lactic acid) (PLLA) nanofibrous scaffolds and this supports neurite outgrowth [80].

PLGA is a copolymer of PLA and PGA polymers. It is approved by the Food and Drug Administration (FDA) as a DDS for cancer treatments, cardiovascular diseases, tissue engineering applications, and vaccine developments because PLGA can be hydrolyzed into monomers (lactic acid and glycolic acid) which are easily metabolized by the human body. It has been widely used in NTE applications due to its excellent properties such as

biodegradability, nontoxicity, film forming ability, swelling, degradation rate, deformation, and permeability. The controllability of the PLA:PGA ratio is particularly important in the development of DDS and conduits for nerve regeneration [32,57,81]. For instance, Schwann cells seeded multichannel PLGA scaffolds demonstrated that PLGA scaffolds have synergistic effects on neural regeneration, but more research is required to promote functional recovery [82,83]. Additionally, PLGA plays a crucial role in the delivery and transportation of therapeutic agents across the blood–brain barrier (BBB). Normally, the exchange of substances across the BBB between the brain tissue and blood is limited due to both physical and metabolic barriers. Therefore, BBB poses a major barrier for drug delivery to the CNS. However, it has been observed that PLGA nanoparticles (NPs) modified with distinctive design features can easily cross the BBB and enhance CNS activity [84]. Although modified PLGA has many advantages, unmodified PLGA has some disadvantages for NTE. The hydrophobicity and lack of cell recognition sites of PLGA scaffolds are the main problem for cell adhesion onto PLGA [85]. For this reason, further improvements are necessary for an effective outcome. For instance, the surface coating or composite with other polymers like alginate increases drug encapsulation efficiency, biocompatibility, and protein release period [86,87].

PEG is one of the most known high biocompatible polymers used to create synthetic hydrogels in NTE. It is a relatively inexpensive, water-soluble, biodegradable, and linear polymer which is composed of ethylene oxide (EO) units [57,88]. PEG is suitable for hydrogel biomaterial due to its hydrophilic groups. However, one of the main drawbacks is that it cannot be used alone due to its low bioactivity like other natural polymers. Generally, it is combined with other natural or synthetic polymers [89].

PEG-based hydrogels and platforms have been widely used to promote neuronal cell growth which is helpful for surviving, proliferation, and differentiation of neural cells. They also have applications as neural guidance conduits in vitro [90]. The potential of neuronal cell growth on PEG hydrogels for the treatment of CNS injuries is quite high [57,91]. PEG-based hydrogels can be designed and assembled in a variety of two- or three-dimensional (2D, 3D) configurations. However, depending on the design of the hydrogel, neuronal cell behavior is more affected in 3D cultures than in 2D cultures [34,92].

PHEMA is one of the biocompatible, nondegradable, and hydrophilic polymer. When temperatures are low (between −20°C and +10°C), it can polymerise. Normally, pHEMA is a hard and brittle material in its dry form, but the hydrophilic groups in its structure cause it to swell and form hydrogels by absorbing water and similar biological fluids. Although pHEMA is not adhesive or attractive, it has been modified with adhesive protein-derived molecules and shows biocompatibility with neurons in vitro and in vivo. pHEMA has been generally used as hydrophilic sponges to enhance axonal regrowth for spinal cord injuries [93]. The main benefit of methacrylate-based hydrogels is that, by modifying their formulation and surface chemistry, they can be tailored to a specific application in the CNS or PNS. Furthermore, pHEMA hydrogels have important roles in the inhibition of cell death, and in supporting and mimicking environment of the host tissue by 3D structure. In addition, it is applied as nerve guidance conduits in NTE applications [7,57,94].

- Conductive Polymers

NTE requires a network that is essential for electrical signaling between neurons and other cell types, completing adhesion, differentiation, and proliferation of cells. Therefore, electrical conductivity for the nerve regeneration process is the first property sought in neural scaffolds. **Electrically conductive polymers (ECPs)** are ideal biomaterials for electrical signal transmission and facilitate the reconstruction of neural connections by mimicking the neural tissue [57,95].

Conductive polymers (CPs) are polymers with electrons in their backbone, which generally exhibit electrical properties similar to metals and semiconductors [96,97]. Most CPs are manipulated through a doping process which includes adding chemical reactants to oxidize or reduce the systems; thus a charge is transferred from dopant molecules to polymer chains via charge carriers (polarons and bipolarons). ECPs are very interesting polymers for NTE applications because of their tailorable electrical, chemical, and physical features for specific applications. However, inadequate biocompatibility that is related to their suboptimal degradation in vivo is the main problem in the use of CPs for NTE. CPs cause chronic inflammation, and immunogenic reactions which require extra treatments including surgeries [98]. To overcome this problem, CPs can be combined with alternative biodegradable polymers. ECPs frequently used in NTE are polypyrrole (PPy), poly-3, 4-ethylenedioxythiphene (PEDOT), polyaniline (PANI), and carbon nanotubes (CNTs) [99].

PPy is produced from pyrrole monomer by the polymerization process and it is a derivative of polyacetylene. It has the potential to be applied in biomedical applications due to its characteristic features including rigidity, insolubility, biocompatibility, easy synthesis, and environmental stability [100]. Generally, PPy has been used in DDS, neural implants, biosensor coatings for neural probes, and molecular memory devices [32,99]. However, the nonbiodegradability of PPy in the use of NTE is a critical issue, but this could be overcome by combining of PPY with other biodegradable polymers like PCL, PLA, and PLGA. For instance, PPy-coated PLGA electrospun nanofibers increased neurite growth [101]. In another study, the combined PPy-PLA fibers enhanced neurite adhesion, elongation, and alignment. Furthermore, PPy-PCL films encouraged cell proliferation and enhanced neurite outgrowth through electrical stimulation both in vitro and in vivo [102]. PPy has not only been combined with synthetic polymers, but also with natural polymers to design three-dimensional electroconductive hydrogels intended to promote healing in traumatic brain injuries or stroke. For example, electrically conductive HA hydrogels incorporated with PPy are produced to support differentiation of human neural stem/progenitor cells (hNSPCs) [103]. In addition to all these, the use of PPy as electrode material in long-term implanted neuroprosthetic devices and becoming a vehicle for localized drug delivery to the CNS by the help of electrical means explains the role of PPy in NTE applications [104].

PEDOT is one of the polythiophene derivatives which is relatively new; it is a versatile and has higher electrical conductivity, thermal and chemical stability than the others [105,106]. PEDOT has been used in NTE for neural electrical stimulation and recording in implantable neural prosthetics devices [17,107]. In addition, PEDOT has recently been involved in the development of structures that enable NSC differentiation via electrical stimulation [108,109].

PANI is another versatile conducting polymer which has many attractive features (i.e., low cost, easy processability, and antibacterial effect) [110]. Like PPy, suboptimal biocompatibility is the main problem for the use of PANI in NTE applications. Therefore, PANI is generally combined with more suitable biodegradable polymers (i.e., PCL/Gel and PLA/PCL) to reduce possible inflammations or immunogenic reactions. When, the nerve stem cells (NSCs) were seeded to PANI-PLA/Ge scaffold, cell attachment and proliferation and neurite outgrowth of NSCs enhanced. Therefore, PANI-PLA/PCL based scaffolds offer great potential for nerve

regeneration and may be used as an effective nerve graft material [111]. Similar to PPy, PANI-based hydrogels have been used as substrates for NSCs differentiation in peripheral nerve regeneration for NTE [112]. Although PANI has many advantages, its use as neural interfaces and biomaterials is less common than PPy and PEDOT, due to its poor cell adhesion and growth properties [113].

CNTs are qualified by electrical properties, remarkable thermal conductivity, and mechanical properties, and they have great potential in NTE applications because of their characteristic features including biocompatible, nonbiodegradable, and conductive properties. They can be functionalized for supporting neural signal transport and enhancing dendrite adhesion and elongation. In NTE, single walled carbon nanotubes (SWCNTs) are used as substrates that modulate and bring to life neural cells in healing by altering the conductance of neurological and brain-related injuries. Studies show that SWCNTs promote neurite outgrowth in vitro [114–116]. Otherwise, multiwalled carbon nanotubes (MWCNTs) have been applied as 3D printed scaffolds for peripheral nerve regeneration [117], targeted delivery systems [118], and developed as artificial neural guidance conduits [119].

7.4 Delivery strategies for growth factor

The use of nerve autografts, allografts, in existing well-established therapies to treat nerve injuries and related diseases is disadvantageous due to insufficient donor nerve resources and a high risk of morbidity, and, in the case of allograft transplantation, an inflammatory host response. In innovative approaches, stem cell–based therapies, alternative strategies to regenerate damaged or diseased nerve tissues, including the delivery of neurotrophic GFs using an appropriate biomaterial, are the focus of NTE's research [120].

Nanotechnology is concerned with the research, understanding, and application of nanoscale materials. In recent years, innovative approaches as well as basic sciences have contributed to the development of interdisciplinary fields initiated by nanotechnology. Recent advances in neuroengineering processes using various types of 2D and 3D nanomaterials (Fig. 7.4) suggest promising results for neural regeneration [121]. Instead of designing tissue regeneration with only cells and scaffolds, the use of exogenous GFs to initiate the regeneration process will contribute to successful results. Some GFs are needed to repair damaged tissue. However, when the bioactivity of GFs is injected into the body in a soluble form, they are retained for a short time at wound sites due to their in vivo enzymatic

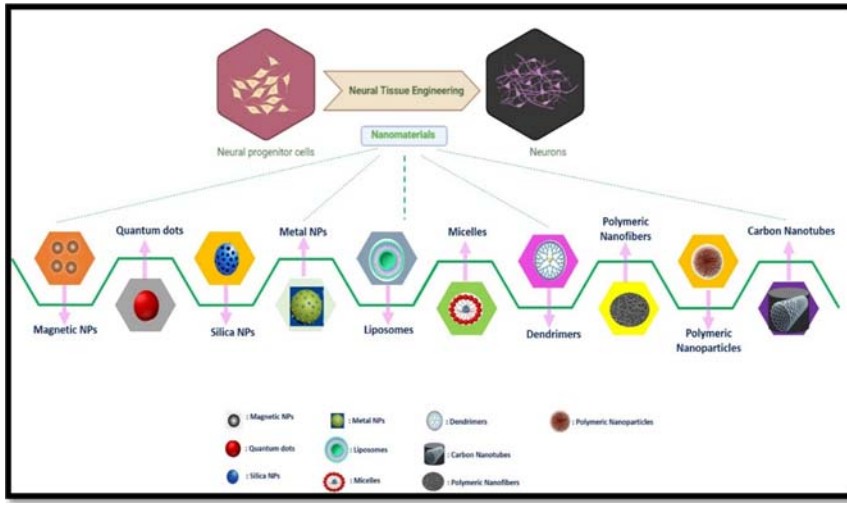

Figure 7.4 Nanomaterials for NTE [121].

and thermal degradation. Embedding and/or fixation of GFs in scaffolds can prevent direct contact of GFs with body fluids, limiting their diffusion, thereby prolonging in vivo activity [122]. Homogeneous secretion of GFs and the use of nutrients facilitate therapeutic effects and cell survival. Because factors released from transplanted cells modulate inflammation, microspheres may degrade within a few days following implantation, allowing implanted cells to migrate to a more permissive environment [123].

7.4.1 Nanoparticles

Nanoparticles (NPs) is a term used for materials that are at least less than 100 nm in size. Generally, depending on the shape, these particulate materials can be 0D, 1D, 2D, or 3D [124]. Polymeric nanoparticles are one of the most studied subjects in modern medicine, as they have a formulation with long circulation times, which can deliver the drug in a controlled mode and at a specific site [125]. Polymer NPs are recognized as a versatile drug delivery system for brain-targeted release, with their ability to protect therapeutic agents, efficiently deliver them to damaged areas, and cross the blood–brain barrier [57,126].

Chitosan NPs are widely used as drug carriers, because they are biodegradable, excellently biocompatible, functionalizable, antibacterial properties, nonimmunogenic, stable, and inexpensive [4,127]. Razavi et al. biofunctionalized the inner surface of the electrospun PLGA nanofibrous channel with laminin containing brain-derived neurotrophic factor (BDNF) and gold nanoparticles (AuNPs) in chitosan NPs. They found that this system can enhance axonal regeneration in the peripheral nerve using

some exogenous factors (i.e., BDNF, AuNPs, and ADSCs in the fibrin matrix) [128].

7.4.2 Nanofibers

Nanofibers (NFs) are nanostructures obtained by electrostatic forces with micro- to nanometer range diameters and high surface area-to-volume ratio [129]. NFs have unique advantages such as mimicking of ECM, tunable porosity, flexible surface functionalization, ability to serve as exclusive platforms for drug delivery applications, and decreased initial burst release [130]. Important work is underway to improve the performance of nanofibers that are produced via electrospinning technique (NFPs) for NTE in vitro or in vivo. Because NFPs have a versatile and functionalized surface, drugs or biomolecules can be surface bound, improving nerve compatibility, cell-platform interactions, and also provides a favorable microenvironment for neurite outgrowth and nerve regeneration, which shows significant advantages over biological cues and single-material scaffolds [131]. Among the various cellular scaffolds for reconstructing neural tissue, biomimetic nanofibrous scaffolds are among the suitable candidates by precisely controlling morphology and shape [27].

Patel et al. developed aligned and bioactive nanofiber scaffolds by immobilizing extracellular matrix protein and growth factor on nanofibers that simulate the physical and biochemical properties of natural matrix fibrils. Basic fibroblast growth factor (bFGF) and laminin were able to bind heparin, enabling their immobilization on the surface of the poly(L-lactic acid) (PLLA) nanofibers. They found that the immobilized biochemical factors synergized with the aligned nanofibers to promote high-efficiency neurite outgrowth, but had less effect on skin cell migration [132].

7.4.3 Hydrogels

Hydrogels, which resemble biological tissues and organs in various ways, are in the class of highly hydrated soft polymeric materials [133,134]. Using hydrogels in TE and biomedicine gained importance when Zhao et al. proposed a hydrated hydroxymethyl methacrylate (HEMA) network for the preparation of contact lenses in the 1960s [15]. Natural or synthetic materials are used in hydrogel production. Although complex ordered structures are easily produced with natural materials, it is not easy to design them in synthetic hydrogels due to the presence of large amounts of water [133,134]. Triggering various environmental factors such as temperature,

pH, ionic strength, and physicochemical interaction is used to fabricate hydrogels to obtain superior mechanical strength by photopolymerization, enzymatic reactions, and other chemical cross-linking [135,136]. The gelation of hydrogels is able to be engineered through cross-linking methods in order to develop dynamically and obtain precise shape, structure, and architecture of hydrogels with using advanced chemical techniques. Thus, hydrogels are able to be customized with attractive properties such as mechanical compatibility with biological tissues, excellent biocompatibility, and controllable degradability [15,137]. In literature, hydrogels have been already used to control the release of NGF, BDNF, GDNF, NT-3, insulin-like growth factor 1, FGF, epidermal growth factor (EGF), as well as VEGF [35,138].

A second pathology, accounting for two-thirds of all ischemic strokes, causes a large volume of striatum and a larger tissue space that extends through the cortex. Ghuman et al. improved the compaction and dispersion of the microspheres using a suspension in an ECM hydrogel. Therefore, the feasibility of introducing microsphere-encapsulated NSCs and endothelial cells (ECs) into a large tissue space was evaluated to determine their effectiveness in supporting cell survival [123].

The impulse of endogenous neural stem/progenitor cells (NSPCs) by GFs for stroke treatment is an encouraging strategy for tissue regeneration. EGF increases proliferation of endogenous NSPCs when delivered directly to the ventricles of the brain; nevertheless, this technique is highly invasive. Cooke et al. have prepared minimally invasive hyaluronan and methylcellulose (HAMC) hydrogels EGF or poly(ethylene glycol)-modified EGF (PEG-EGF) hydrogels. They showed that PEGylation reduces the rate of EGF degradation by enzymes, resulting in a momentous increase in protein aggregation at greater tissue depths, increased NSPC proliferation in uninjured/stroke-damaged brains, and significantly increased NSPC stimulation in stroke-damaged brains [139].

Cai et al. developed a macroporous hydrogel with a guide channel in which a photosensitive phenyl azide monomer is copolymerized with pHEMA. Combined with its function of sustained release of bFGF, the collagen-modified hydrogel channel will provide the appropriate microenvironment to support cell adhesion and survival. They expect that scaffold is a suitable biological niche for neuronal regeneration [140].

7.4.4 Microneedles

Microneedles (MNs), which consist of micron-sized needles arrayed on a small patch, have been the focus of attention by various researchers for transdermal drug delivery due to their rapid onset of action, improved permeability, better patient compliance, and self-administration. The biggest problem with transdermal technology is that most drugs do not pass through the skin at the speed necessary for therapeutic effects. Administration of drugs using MN lets drug molecules pass through the stratum corneum layer and thus more drug molecules enter the VEGF, whose use is limited due to inhomogeneous VEGF distribution and limited VEGF retention in the brain after intracranial/intravenous injection, Liu et al. developed a gelatin methacryloyl (GelMA) MN-based platform for controlled VEGF local delivery. AAV-VEGF-loaded MNs in adult rats, in which they modeled ischemic stroke, were placed epicortically. Eight weeks later, they evaluated angiogenesis and NSC proliferation and migration and noted that GelMA MN implantation did not elicit an obvious inflammatory response and was biocompatible. Liu et al. reported that MNs loaded with AAV-VEGF increase VEGF expression and increase functional angiogenesis and neurogenesis [30].

7.4.5 Carbon-based materials

Electrical signals are important in neurons and their activities. Graphene is a useful material for NTE because of its good electrical properties. To use hMSCs for nerve regeneration, it is important to enable cells to differentiate into neurons rather than glia [141]. Electroconductive materials offer an exciting basis for developing biomaterials for the regeneration of neural tissues [142]. Thanks to the carboxylic groups on GO, it can covalently bind to biomolecules and GFs to increase cell proliferation and differentiation, and allow surface modification, enabling the development of composites for different applications in TE. While there have been some reports of dose-dependent cytotoxicity of GO, other articles report that if GO sheets are coated with polymers, they will no longer be toxic [120,143].

Park et al. demonstrated an electrical excitation on cells different from hNSCs using graphene as a transparent electrode that enhances the differentiation of hNSCs into neurons. They noted that laminin molecules adhere to both graphene and glass and aid in hNSC binding. Moreover, by proliferating in a culture medium with essential bFGF and EGF, hNSCs differentiated for a long month in a culture medium without bFGF and

EGF. Thus, they showed that graphene has a great surface property to use in neuroscience [144].

7.5 Conclusion

Every cell in our body turns into a certain cell type (i.e., muscle, nerve cell, etc.) at the end of cellular differentiation for a purpose. Nevertheless, neurons are thought to lose their division properties due to the unknown factors. Thanks to NTE, it is trying to eliminate the damage caused by tissue scaffolds made to the relevant area after injuries to neurons. Instead of using these complex tissue scaffolds made of natural and synthetic polymers alone or a composite of them (blending of natural and synthetic polymers) and carbon-based nanomaterials, the use of growth factors that will accelerate the healing process of the damage is becoming increasingly important. In the literature, various strategies are determined in order to deliver NGFs and they are classified into five strategies as nanoparticles, nanofibers, hydrogels, carbon-based materials (i.e., graphene, GO, and CNTs), and microneedles. Hydrogels are mostly used as delivery vehicles of NGFs due to their good mechanical properties and the dynamic shape, structure, and architecture of hydrogels can be produced easily. In the future new strategies that are to be developed, researchers are focused on improving delivery capacities and controlled release of NGFs for the healing of neuron injuries.

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