CHAPTER 12

Characterization of scaffolds for neural tissue engineering

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12.1 Introduction

The human brain is a mysterious source of knowledge and to understand its functioning mechanisms, it is necessary to study the intricate relationship with the many elements of the central nervous and peripheral systems. This knowledge is essential for investigating the reasons for many neural disorders and for developing reasonable treatments for metabolic, ischemic, degenerative, or congenital diseases of the central or peripheral nervous systems [1]. The central nervous system (CNS) and the peripheral nervous system (PNS) are the two major components of the nervous system. The brain, spinal cord, ocular, olfactory, and functional systems make up the CNS. It analyses and transmits signals to the PNS. It is made up of cranial nerves that grow from the brain, spinal nerves that grow from the spinal cord, and sensory nerve cell bodies. The spinal column receives sensory and excitatory signals from peripheral nerves, which then convey those signals to the muscles [2]. Neurons and neuroglia are the two types of cells that comprise the nervous system (Fig. 12.1).

Neurons, which contains the cell body and its extensions, are the principle morphological and physiological components of the nervous system (axons and dendrites). Ganglia are clusters of sensory nerve soma situated slightly outside the spine. Dendrites carry electrical impulses to the neuron cell body, while the axon carries them away. Glial cells, also known as neuroglia, are support cells that aid in the function of neurons. They include Schwann cells in the PNS and astrocytes and oligodendrocytes in the CNS. Glial cells are more plentiful than neurons, and unlike neurons, which cannot divide through mitosis, glial cells may divide to some extent [3]. All axons in the PNS are encased in live Schwann cell sheaths. The

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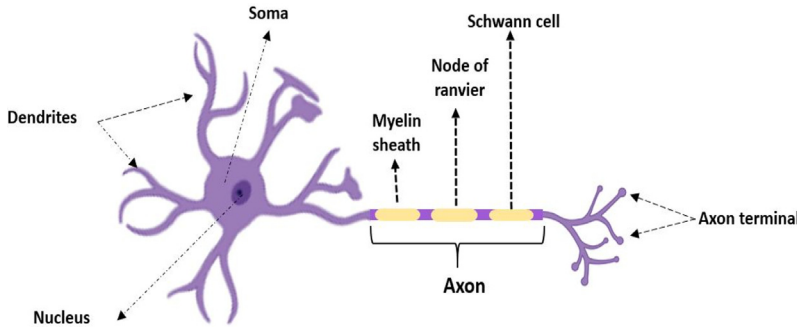

Figure 12.1 Schematic illustration of the nervous system.

neurilemma, a basement membrane resembling that seen in epithelial layers, is present on the outside of this Schwann cell layer. CNS axons lack the basement membrane and sheath of Schwann cells, in contrast to those in the PNS. Instead, a myelin coating that acts as insulation surrounds many axons. Myelin makes it possible to speed up the transmission of nerve impulses, which is crucial for long-distance axons (up to 1 m) [3].

The fundamental cell type in the nervous system, the neuron, is in charge of transmitting and receiving messages via action potentials. The neuron is made up of four morphologically distinct components: a cell body or soma, one axon (a nerve process) that extends from it, presynaptic terminals, and dendrites. Functionally, neurons can be divided into sensory or afferent neurons, interneurons, and motor or efferent neurons. Neurons can be structurally categorized as multipolar (one axon and many multi-branched dendrites), bipolar (one axon and one dendrite), and pseudou-nipolar (two axons: peripheral and central) neurons. The CNS receives information from the afferent neurons. Motor or efferent neurons convey the message from the central nervous system to the muscles and glands [4]. The other kind of cell in nervous tissue is called neuroglia, which is smaller and more prevalent. In both the CNS and PNS, neuroglia provide the supporting cells for the soma, axons, and dendrites of neurons [5].

Schwann cells (SC) surround the neuron axon in the PNS glia, while perineuronal satellite cells surround the neuron cell body. By encircling a short segment of the axon, the SC creates myelinating axons. This process results in the formation of the myelin sheath, a brilliant white protective covering surrounding the axon. SC is also present on unmyelinated axons. In the PNS, satellite cells, which are flat cells, surround and sustain the cell bodies of neurons. Both SC and satellite cells are positioned in the

interneuronal space between neurons, where they engulf and separate unmyelinated axons [6].

Glia in the CNS is mainly composed of ependymal cells, microglia, oligodendrocytes, and astrocytes. Astrocytes are the cells responsible for controlling the extracellular environment of the brain, helping to construct the blood–brain barrier, and holding capillaries, neuron cell bodies, and dendrites in place. They are also responsible for repairing injured brain tissue. Astrocytes also make communication with the ependymal cells of the ventricular arrangement. The PNS that creates and maintains the CNS myelin is equivalent to the SC's oligodendrocytes. The immune system's reaction and the preservation of homeostasis are both regulated by microglial cells. Ependymal cells play a role in checking the production and circulation of cerebrospinal fluid [7]. Glial cells support and protect the CNS's neural networks structurally, lead to the migration of new neurons to the right location, and promote the expansion of their axons. Additionally, they create myelin sheaths, trophic and growth factors, and plasticity that isolate the axon and help accelerate the propagation of action potentials in the nervous system. Microglia eliminate debris created after injury or cell death. In order to heal damaged neural tissue, astrocytes operate as a bridge to transmit nutrients to neurons and multiply to form astrocyte scars (reactive gliosis) [8].

Millions of individuals worldwide suffer from damage to the nervous system. These accidents frequently result in chronic cognitive, motor, or psychiatric disabilities in young individuals [9]. Due to protease activity and the depletion of the nerve cell bodies' metabolic resources, when a nerve is altered, the altered section starts to deteriorate. The cytoskeleton degrades first, followed by the breakdown of the cell membrane. Although the proximal end of the nerve swells, retrograde degradation only causes minimal injury. Myelin lipids are released by Schwann cells that surround distant axons after the cytoskeleton and membrane have degraded. Macrophages and Schwann cells, which are phagocytic cells, scavenge myelin and axonal debris. These cells also generate cytokines that promote axon development. Axons must grow longer until they reach their distant goal during functional reinnervation. Since axon regeneration in humans happens at a pace of about 2–5 mm per day, serious wounds can take months to heal [3].

An additional stage of regeneration is needed when a hollow nerve canal is employed to heal a damaged peripheral nerve. Following damage, a fibrin bridge develops along the canal and across the area of the defect.

Macrophages and other cells that are expected to contribute to the debris removal process are part of this fibrin bridge. The fibrin bridge retracts and normal regeneration takes place when Schwann cells and capillaries begin to appear across the cavity [3]. The patient's life is severely and persistently affected by damage to the central nervous system (CNS). This is due to the fact that brain function frequently is not fully restored. Axonal damage is frequently caused by injury. Axons' capacity for regeneration is also constrained, despite the fact that the central nervous system contains a number of mechanisms for repairing damaged neural circuits. It's critical to understand these pathways in order to create fresh CNS damage treatments [10].

The most common form of treatment for peripheral nerve damage is autologous nerve grafting or direct end-to-end surgical reconnection of injured nerve terminals. The two nerve terminals can be stitched together to fix any small gaps or defects in the nerve. This strategy is not applicable for longer nerve spaces because any stress placed on the nerve cord would prevent nerve regrowth. Therefore, an autologous nerve graft from another area of the body is utilized to spread the damage location for a bigger neural deficit. The loss of function at the donor location and the requirement for many surgeries are drawbacks to this approach [11]. The clinical outlook for CNS damage is less encouraging. To lower the risk of subsequent damage, surgery may be performed if there are bone fragments close to the injury site. To lessen swelling and further damage, antiinflammatory medications such as methylprednisone are frequently administered. Unfortunately, there is no available cure at the moment for regaining nerve function. Patients start a protracted recovery process when the injury's swelling reduces [12].

12.2 Neural tissue engineering

It is well recognized that the latest improvements in tissue engineering present the most practical method for repairing neurological problems and have a broad variety of practices in regenerative medicine [1]. The ECM, which mostly consists of polysaccharides and proteins, controls cellular activity by impacting cells with biochemical signals and topographic cues. The nervous system's healing procedure is intricate. A different approach to neural regeneration is provided by tissue-engineered scaffolds. The potential scaffolds for neural tissue replacement might be submicron and nanoscale fibrous structures that resemble the extracellular matrix (ECM) in nature [13]. The applications for neural tissue replacement have been researched

using a variety of biomaterials, including hydrogels, polymers, peptide nanofibers, and different aligned materials [14]. The growth factors and cells were also added to the scaffold in several studies to expedite nerve regeneration, and the outcomes showed the effectiveness of the strategy [15].

12.3 Ideal properties of scaffolds for forming neural scaffolds

By providing support for the surviving neurons around the injured region, releasing trophic components, and guiding axonal development, tissue-engineered scaffolds can be created to allow the regeneration of damaged neural tissues [16]. The scaffolds for nerve regeneration should have suitable mechanical possessions to mimic the ECM [1] (Fig. 12.2).

For the optimal neural tissue scaffolds, the following factors should be taken into account while choosing biomaterials [9]: The skill of cells must adhere to the scaffold, function, progress, and proliferate. In order to create biomimetic materials, the scaffold's surface can be modified utilizing bioactive chemicals. Biomaterials may be coated with bioactive molecules such as short peptide sequences, laminin, fibronectin, vitronectin, and long ECM protein chains. Enhancing the surface modification encourages cell

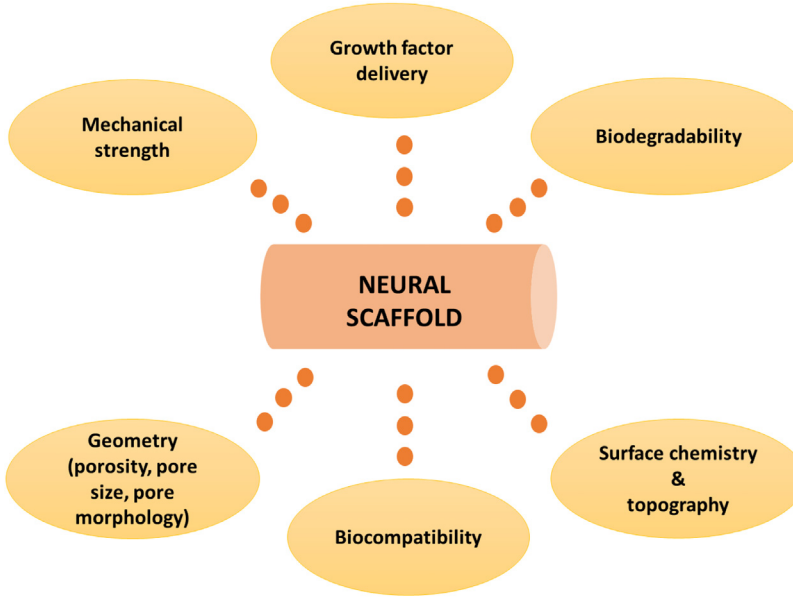

Figure 12.2 The required possessions of the scaffolds for neural tissue replacement.

proliferation and adhesion. Surface characteristics like hydrophilicity and charge density affect how well cells adhere to surfaces. Cell attachment, growth, and proliferation on the scaffold are significantly influenced by toxicity profiles in addition to biocompatibility [17]. Producing biodegradable scaffolds has a number of benefits, one of which is that they do not require surgical removal since they are absorbed by the body's surrounding tissues. Controlled biodegradable scaffolds are created in neural tissue engineering because they are designed to assist the proliferation of nerve cells before being dissolved by the body while healing is taking place. Additionally, the biodegradable scaffold will make it easier for nearby cells to make their own extracellular matrices. It is important to take precautions to make sure that any byproducts of biodegradation are nontoxic and simple to discard. Neuroma formation can also be induced by cytotoxic actions. The reduction of chronic inflammation ought to be aided by biodegradability [18]. To replicate real tissue, a scaffold should have the right shape and porosity. The diffusion of growth agents and nutrients into the scaffold and surrounding tissue is necessary for vascularization and requires the proper pore size. For cell attachment, diffusion of nutrients into cells and waste products, porous structures must be produced [17]. Additionally, mechanical characteristics have a significant influence on the implantation of tissue in the injured region. In terms of mechanical properties, polymeric nanofibers are particularly favorable. They are known for possessing exceptional mechanical characteristics including tensile strength, shear modulus, and modulus of elasticity. Recent results demonstrate that these characteristics rise as the fiber's diameter decreases, which can be attributed to the decrease in the number density of intrinsic defects inside materials. These particular mechanical characteristics enable the control of cell function and provide scaffold with the ability to withstand the stresses applied by cytoskeletal components. Therefore, the creation of an ideal scaffold is mostly determined by the optimum mechanical properties and porous scaffold architecture, which together play a crucial role in promoting cell infiltration and vascularization [19].

12.3.1 Natural polymer-based scaffolds

Natural polymers have been commonly employed as alternative bioengineering materials due to their natural origin. Tissue rejection in natural polymers is low since their chemical composition and structure can be detected by host cells. Excellent cell adherence is provided by these

polymer types [4]. The primary element of connective tissues, which gives the body its structure and support, is collagen. It is the only biopolymer that has been clinically proven to be useful as nerve tissue. This polymer is also combined with proteins and other polymers. Collagen-based nerve guide structures have been utilized in neural tissue engineering to repair injured nerves [20]. However, as collagen absorbs water, it loses some of its mechanical and structural stability [9]. Another natural polymer gelatin is a denatured protein and it has been utilized to create neural tissues to treat neural disorders [21]. All vertebrates naturally contain branched glucosamines including hyaluronic acid. Because it encourages neurite outgrowth, it has risen in importance in neural tissue engineering. The PNS and CNS of HA-formed hydrogels promote cell proliferation and survival rates of neural precursors [22,23]. A linear polymer called alginate is produced by bacteria and brown algae. Alginate can be combined with other polymers to create scaffolds that have a great deal of potential for helping to regenerate peripheral nerves and cure spinal cord injury [24]. Chitosan, a structural polymer found naturally in crustaceans and shellfish, is transformed into the carbohydrate chitosan by chemical deacetylation. Cell contact, cell attachment, and neurite outgrowth are all supported by chitosan hydrogels [25].

12.3.2 Synthetic polymer-based scaffolds

Another scheme for neural tissue engineering is the use of synthetic biomaterials. Poly-e-caprolactone (PCL), poly-L-lactic acid (PLLA), or poly-D, L-lactic-co-glycolic acid (PLGA) are examples of synthetic polymers that are often utilized. These materials can compensate for the missing aspects of natural biomaterials with their adaptable structures according to the tissue type. In addition, these materials are advantageous in controlling degradability. These polymers can take various forms such as aligned fibers and tubular structures. However, they need to be supplemented with natural polymers in terms of bioactivity [26]. PLLA in the form of foam can facilitate structural and functional in vivo regeneration [27].

A conventional polymer used in electrospinning processes is PCL. To increase its biocompatibility, cell adhesion qualities, and encourage nerve fiber regeneration, PCL can be mixed with natural polymers or coated with cells [28]. To create fibers with tubular structure lumen that promote cell adhesion, migration, and proliferation, PLLA has been employed [29]. Synthetic polymers including PCL or PLGA have shown effectiveness in

nerve remodeling conduits and medication delivery microparticles [30]. It has also been demonstrated that other synthetic polymers, such as poly(acrylonitrile co-methyl acrylate) (PAN-MA) and polydioxanone (PDS), may be used to stimulate neuron development. These synthetic materials promote the development of brain cells, although their capacity to promote cell survival and proliferation varies as a result of their unique characteristics [13]. Table 12.1 lists the benefits and drawbacks of biological materials that are natural and manufactured.

12.3.3 Electrically conductive polymers

Electrical stimulation triggers a response from nerve cells and communication can be established through conductive polymers [31]. The creation of composite scaffolds comprising synthetic and natural polymers as well as the application of cells like Schwann cells, stem cells, or growth factors to the scaffold's surface are two methods for creating biochemical cues. Electrical cues are essential for controlling the growth of axons and neurite outgrowth because neurons are engaged in electrical signal transmission [9].

Polypyrrole (PPY) and carbon nanotubes (CNTs) are two examples of commonly utilized conductive polymers [32]. PPY is a polymer formed by the polymerization of pyrrole monomer. When combined with other polymers, CNTs show accelerated neurite outgrowth and elongation in all directions [31]. To improve neurite outgrowth, PPY has also been combined with various synthetic polymers such as PCL, PLA, and PLGA.

12.4 Methods for fabrication of neural scaffolds

The creation of several strategies to produce complex structures from diverse natural and synthetic components has led to the production of tissue-engineered components, including scaffolds and integrated systems. The goal has always been to construct the best scaffolds possible that are compatible with the mechanical, chemical, and physical characteristics of the tissue. For their use in nerve tissue engineering (NTE), these scaffolds must possess a number of desired characteristics, including pores, fibers, and channels. The most prominent biofabrication techniques for creating NTE structures are identified and described in this section. Each of these techniques has its own benefits and drawbacks and may generate various forms and structures to satisfy the demands and requirements of the particular application.

Table 12.1 Biomaterials for the replacement of neural tissue, both natural and synthetic.

Materials	Advantages	Disadvantages
Collagen	Good biocompatibility High mechanical strength Biodegradability Low antigenicity Good water-uptake	Lack of cell integration Low mechanical and structural stability
Gelatin	Biocompatible Proper mechanical property	Lack of cell integration
Hyaluronic acid (HA)	Biocompatible proper mechanical property	Low degradation rate
Alginate	Biocompatible proper mechanical property	Inducing inflammatory response
Chitosan	Biocompatible proper mechanical property	Low degradation rate Lack of cell integration
Alginate	Biocompatibility low cost Low immunogenicity	Low stability Lack of binding sites
Hyaluronic acid	Easy to produce and modify nonadhesive Biodegradable	Poor mechanical strength High degradation rate
Poly-ε-caprolactone (PCL)	Biocompatible High mechanical property	Low degradation rate
Poly-L-lactic acid (PLLA)	Biocompatible Biodegradable	Slow degradation rate
Poly-D, L-lactic-co-glycolic acid (PLGA)	High mechanical property Biocompatible Biodegradable	Lack of cell integration
Polydioxanone (PDS)	Biodegradable It has shape memory Great strength	Stiff Low reactivity
Poly(acrylonitrile co-methylacrylate) (PAN-MA)	Good strength and modulus High toughness	Lacks drawability and has a low heat treatment endurance as a result of interactions between nitrile groups

Continued

Table 12.1 Biomaterials for the replacement of neural tissue, both natural and synthetic.—cont'd

Materials	Advantages	Disadvantages
Polypyrrole (PPY)	High electrical conductivity Flexible Excellent environmental stability Low toxicity Good cell adhesion and growth under electrical simulation	Deterioration of conductivity in the presence of current

12.4.1 Electrohydrodynamic techniques

The foundation of electrohydrodynamic manufacturing techniques is the electrostatic attraction of a liquid that is exposed to an electric field as it exits a nozzle and then collects on a plate. Three main parts are often used in these manufacturing setups: (i) a power source with a high voltage, often in the kilovolt range (ii) a metallic-needled syringe (spinneret), and (iii) a grounded collector. A viscoelastic solution is injected in the syringe in a conventional electrohydrodynamic method. As a tiny droplet is squeezed out of the tip, an electrical potential difference is applied between the droplet and the plate, causing the droplet to become charged. The positive electrode of the high-voltage power supply is connected to the metallic needle, and the negative electrode is connected to the grounded conductive collector, generating the electrical field. The surface tension of the fluid begins to be overcome by electrostatic repulsion as voltage rises, and the suspended droplet deforms into a conical droplet known as the Taylor cone. When the surface tension is eventually defeated by electrostatic repulsion, an intense, charged jet of solution is expelled from the needle's tip in the direction of the grounded collector [33]. The solvent is expelled as the material travels to the collector, and when the procedure is through, nano- or microstructures are produced. Electrospinning and electrospraying are two distinct electrohydrodynamic techniques that have been developed. For the creation of nanoscale and microscale fibers and particles, electrospinning and electrospraying are two extremely flexible and scalable electrohydrodynamic techniques. Liquid atomization by electrical forces is called electrospraying. The concentration of the solution is the primary

distinction between electrospraying and electrospinning. Due to the low concentration of the solution in electrospraying, the jet becomes unstable and small droplets develop as a result. In order to avoid droplet aggregation and coagulation, these highly charged droplets self-disperse in space. Additionally, when the solvent evaporates, the droplets contract and solidify, leaving behind solid particles that are deposited on the grounded collector. The kind of the collector is critical for achieving aligned orientation in electrospinning. In the past, a variety of specially constructed collectors, such as rotational drums, metal frames, or two conductive substrates separated by an insulating gap, have been employed to produce aligned electrospun fibers. The resultant nanofiber structure is also impacted by the collector's form. The deposited patterns can be constructed of various materials, such as copper, aluminum, gold, wood, and so on, and various geometries including circles, triangles, squares, crosses, rectangles, etc. Additionally, a rotating mandrel can be paired with a liquid system with a coagulation bath in lieu of a collector to produce continuous yarn from electrospun fibers. In general, mechanical, magnetic, or electrostatic methods can be used to achieve the required fiber alignment [34].

12.4.2 3D printing approach for nerve regeneration

Recently, 3D bioprinting techniques have been discovered, which simplify the organization of the size and form of 3D scaffolds and develop cell-filled scaffolds. Rapid prototyping (RP) technologies are used in 3D bioprinting, also known as additive manufacturing (AM), to print biomaterials such as cells, growth factors, and other biomaterials in layers. This technology allows for the creation of biological structures that closely resemble the characteristics of actual tissues and organs. Along with 3D modeling program, an X, Y, and Z-axis drive mechanism, as well as bioprinting materials and inks, are often included in a 3D bioprinting system. First, CAD or CT pictures are used to construct a design. Then, a computer-attached bioprinting apparatus creates a 3D scaffold structure [35]. Bioink is a critical component of 3D bioprinting. The biomaterials that makeup bioinks can be utilized to include biomolecules and encapsulated cells. Prior to printing, it is crucial to take into account a bioink's basic characteristics, such as cross-linking, viscosity, and gelation. These characteristics have an impact on morphology, cell viability, and proliferation [36]. The operating principles outline three fundamental 3D bioprinting technologies: extrusion, inkjet, and laser-assisted. Inkjet printing is a 3D method that can lay down tiny

droplets of polymer solution on the substrate one layer at a time in a controlled way along the x, y, and z axes. To create droplets and a three-dimensional clump of material composed layer by layer, the ink material must continue to be in a liquid state. Compared to other printing techniques, the deposition volume is less to produce prints with higher print resolution [37]. In extrusion-based bioprinting, the structure is built layer by layer through the moveable nozzle of the extrusion print head while being controlled by a computer. It is separated into fusion-based bioprinting and 3D plotting. Inks are often polymers, slurries, or dispersions, the majority of which are viscous materials [36]. The photopolymerization theory of the photosensitive liquid resin is used in the stereolithography technique. The liquid resin hardens to the surface from the place where the light spot is scanned when the computer-controlled laser beam scans the resin liquid surface while being reflected by the reflecting mirror. The lift platform lowers the platform to one layer height once the first layer sweep is complete. Once it has a complete 3D scaffold, it scans the following layer. SLA is a sluggish and inconsistent printing method. Photosensitive viscous polymers are the components employed in this method [32]. The printing method known as digital light processing (DLP) also uses resin photopolymerization. The main distinction between the SLA and DLP is that the DLP features a digital micro-mirror device (DMD) made up of millions of mirrors, whereas the SLA projects the laser beam's light spot [36].

12.4.2.1 Other methods

Spinal cord axon regeneration has been demonstrated to be supported by the fabrication of a multichannel scaffold utilizing injection molding and the solvent evaporation method. The preparation of nerve guides is also thought to be acceptable using melt compression and melt extrusion. Multichannel biodegradable nerve guides are manufactured using cutting-edge fabrication techniques such as the wire mesh method and the mandrel sticking method without the need for complicated equipment, acidic environments, or exposure to high temperatures. By regulating the alignment of Schwann cells, micropatterning, a novel patterning method for biodegradable polymers, is claimed to improve peripheral nerve regeneration. New fabrication methods are continuously being developed to deliver the ideal nanostructure topography for sufficient nerve development [1].

12.5 Characterization techniques of scaffolds

The challenge of delivering electrical signals for directed neuron development over a flexible polymeric neural scaffold was investigated by Gupta et al. Using an electric field alignment approach, aligned multiwalled carbon nanotube (MWCNT) chitosan scaffolds were fabricated. The chitosan MWCNT solution was made and equally divided into two glass molds, one of which was placed in a 220 V AC electric field and the other of which was not. They spent the night in a hot air oven set to 80°C. In order to prevent any impacts of acetic acid on the cytocompatibility of the films, both manufactured films were collected and stored in a hot air oven at 135°C for 6 h. Their findings show that by using an external AC electric field during synthesis, a significant chunk of CNTs was effectively aligned in chitosan. Chitosan-aligned MWCNT scaffolds outperformed chitosan-random MWCNTs and pure chitosan scaffolds in terms of strength in the direction of alignment by a factor of 21.92. In comparison to chitosan-aligned MWCNT films and chitosan scaffolds in the transverse direction, the electrical conductivity of chitosan-aligned MWCNT scaffolds in the alignment direction is considerably (100.000-fold) greater. It was discovered that a key aspect supporting the capability of these scaffolds to give mechanical and electrical signals for neural regeneration was the excellent survival of HT-22 rat hippocampus neurons across all scaffolds [38].

In the work of Baolin Guo et al., they produced electroactive degradable porous tubular scaffolds using a solution-casting/salt-filtration technique using mixes of polycaprolactone and hyperbranched degradable conductive copolymer at various feed rates. These scaffolds feature interconnected pores evenly dispersed throughout the cross-section and surface, according to SEM and micro-computed tomography measurements. Depending on the proportion of hyperbranched degradable conductive copolymer to polycaprolactone, films with the same composition as scaffolds have an electrical conductivity range from 3.4 10^{-6} and 3.1 10^{-7} S cm^{-1}. It was possible to create a hydrophilic surface with a water contact angle of around 30 by doping the films with (±)-10-camphor sulfonic acid. Tensile tests were used to examine the films' mechanical characteristics, and SEM was used to examine their morphology. When the scaffolds were put through the WST test (water-soluble tetrazolium salts) to measure cell proliferation and cytotoxicity, they were shown to be non-cytotoxic when used with human epidermal keratinocytes (HaCaT) cell

line. These biodegradable electroactive tubular scaffolds make excellent candidates for brain tissue engineering applications [39].

Electrical polarization may be used to create platforms for brain tissue engineering that are more effective, according to research by Barroca et al. A lab-built corona polarizing device was used to electrically polarize scaffolds made of PLLA and aligned nanofibers. Characterization of the platforms by thermally induced depolarization currents revealed a polarization of 60×10^{-1}°C cm on polarized nanofibers. It has been demonstrated by other in vitro investigations utilizing neuroblastoma cells that platform polarization facilitates retinoic acid-induced neuronal development. Additionally, polar-oriented nanofibers boosted neurite outgrowth by 30% (+70 μm) compared to nonpolar-aligned nanofibers and by 50% (+100 μm) compared to control conditions while differentiating embryonic cortical neurons. Thus, polar-aligned nanofibers have emerged as promising biocompatible and bioactive platforms for neural tissue regeneration due to the interaction between topographic cues and electrical polarization. The produced PLLA polarized nanofiber scaffolds can be employed as therapeutic devices with a long shelf life for neuro repair applications because of their long-term induced polarization [40].

The physicochemical characteristics of pure PCL and PCL/HA nanofiber scaffolds were examined in the study by Entekhabi et al. The findings show that compared to pure PCL scaffolds, a PCL/HA blended structure with an ideal proportion of HA may improve the biochemical, biomechanical, and biological aspects of the scaffolds, and this composite scaffold can better satisfy the needs of neural tissue regeneration [41].

By combining electrospinning and electrospraying methods, Zhu et al. developed a novel tissue engineering scaffold with a highly aligned PCL microfiber framework and adjustable PLGA core-shell nanospheres with embedded bioactive factors. To build a novel, highly aligned, biomimetic neural structure with a continuous bioactive factor release environment and nano/micro characteristics, they combined electrospinning and coaxial electrospinning methods. The created nanocomposite scaffold has an enhanced hydrophilic surface property and a nanostructure suited for the cell. In order to guarantee a consistent release of the bioactive ingredient, the core-shell nanospheres are also inserted into the microfiber scaffold. The findings demonstrate that the nanocomposite scaffold considerably promotes the growth of rat pheochromocytoma (PC-12) cells. The highly aligned scaffold also boosted neurite extension along the fiber and directed neurite length in both PC-12 and astrocyte cell lines, as seen by confocal

microscopy pictures; this shows that the scaffold has the capacity to direct the formation and regeneration of neural tissue [42].

Using electrospun serum albumin (SA) fibrous scaffolds, Hsu et al. created neuronal tissue engineered constructions for a different investigation. Hemin-doped iron-containing porphyrins were functionalized with various recombinant proteins and growth factors to promote cell adhesion and proliferation after being doped with hemin to make the SA scaffolds more conductive. The results demonstrated that their scaffolds could promote the attachment, proliferation, and neuronal development of neural stem cells (NSCs) generated from human-induced pluripotent stem cells (hiPSCs). Additionally, they demonstrated that their scaffolds could integrate active growth factors and gradually release them, changing the behavior of grown cells and eliminating the need. Electrical stimulation on the doped SA scaffold had a positive effect on the development of populations of neurons that had more branching neurites than controls did. These conductive SA fibrous scaffolds have a wide range of applications in nerve regeneration procedures because they encourage cell proliferation, differentiation, and neurite branching of hiPSC-derived NSCs [43].

In their study, Montgomery et al. examined two embryoid body (EB)—mediated procedures for producing neurons from murine iPSCs and ESCs: an 8-day 4-4+ protocol using soluble retinoic acid in the previous 4 days and a 6-day 2-4+ protocol utilizing soluble retinoic acid in the previous 4 days, sonic hedgehog agonist purmorphamine, a tiny chemical, throughout the day. For a further 14 days, EBs were seeded on fibrin scaffolds to allow for the development of EBs into neurons. When compared to the EBs produced by the 4/4+ protocol, the 2/4+ protocol produced a larger percentage of neurons in both iPSCs and ESCs. The outcomes showed that the fibrin-based cell delivery platform could successfully be used with murine iPSCs and that the 2/4+ differentiation process may optimize the number of neurons produced from EBs obtained from murine iPSCs seeded in fibrin. Additionally, these outcomes support additional research into 3D fibrin—based scaffolds as a way to distribute iPSC-derived neuronal cells [44].

Nspet et al. examined at how the activity of rat brain—derived neural stem cells (NSCs) was affected by 3D electrospun PCL scaffolds. The interaction of NSCs with submicron PCL fiber scaffolds with randomly aligned fibers that have an average fiber diameter of 750 ± 100 nm was studied. In order to ascertain if changes in surface tension of fiber scaffolds and amino functionalization had an impact on NSCs' capacity to proliferate

and differentiate, PCL scaffolds were treated with ethylenediamine (ED). Due to the amine moieties found on the surface of the fibers, the fibrous scaffold's surface tension rose after being treated with ED. NSC differentiation was unaffected by surface treatment, but the modified scaffolds exhibited more hydrophilic behavior, which led to a considerable rise in the number of cultured cells and enhanced dispersal throughout the whole scaffold. Stem cells mostly developed into oligodendrocytes when NSCs were planted on PCL scaffolds in the presence of 10% FBS, showing that electrospun PCL has the ability to control the differentiation of NSCs in a particular lineage. The findings of this work can be used to create electrospun biomaterial scaffolds that control the proliferation and differentiation of NSCs in brain tissue engineering [45].

In the work by Haddad et al., electrospun polylactic acid nanofibers were used to create 3D scaffolds with interconnected holes. Wet chemistry was used to functionalize these scaffolds with polyallylamine and insert amine groups. The experimental setup for the amination process was carefully considered and chosen such that a significant number of amine groups could be added while still preserving the scaffold's mechanical and structural qualities. To further activate these amine structures, covalent grafting of epidermal growth factor was then carried out. After that, the scaffolds underwent biocompatibility testing while being cultivated with neural stem–like cells (NSLCs). Their findings showed that NSLCs could develop on these epidermal growth factor (EGF)-grafted substrates and could survive for up to 14 days without media-soluble growth factors [46].

As prospective matrices for nerve tissue engineering and repair, Alhosseini et al. developed electrospun polyvinyl alcohol (PVA)/chitosan nanofiber scaffolds with high pore diameters. The porosity of the scaffolds at different levels of their depth was assessed using an image analysis approach after PVA fibers were changed by mixing with chitosan. The structural, physicochemical, biodegradable, and swelling properties of chitosan nanofiber scaffolds were also assessed. When the chitosan-containing scaffolds were used for in vitro cell culture in contact with PC12 nerve cells, it was found that they had the optimum balance of characteristics to meet the needs of nerve cells. The biocompatibility of PVA scaffolds is increased by the addition of chitosan, and the vitality and multiplication of nerve cells are also increased. In fact, the synthesis of a neurofriendly polymeric mix was proved to be made possible by the addition of modest amounts of chitosan to PVA scaffolds [47].

Hydrogel scaffolds made of fibrin, polyurethane (PU), and MWCNTs were produced for use in neural tissue engineering in the work by Hasanzadeh et al. The acquired results looked at the impact of the PU/MWCNT addition on enhancing the mechanical and electrical conductivity of fibrin gel as well as cell adhesion. The findings showed that human embryonic stem cells (hESC) viability and growth were greater in fibrin/PU/MWCNT hydrogels than in fibrin hydrogels. As a result, the fibrin/PU/MWCNT hydrogel can encourage cell growth and be utilized as a scaffold to create an environment that will boost the viability of the cells. The electrical conductivity and hydrophilicity of PU were increased, while the fiber diameter was decreased, by the addition of MWCNTs. The hydrogel's stiffness was increased, its rate of breakdown was slowed, and cell adhesion and proliferation were sharply boosted by the addition of PU/MWCNTs to the fibrin gel [48].

The interaction of mouse embryonic cortical neurons with randomly oriented electrospun scaffolds made of PLLA and PLGA was examined in the Nisbet et al. research. Different potassium hydroxide (KOH) concentrations were applied to the scaffolds' surfaces in order to partially hydrolyze the surface and vary the surface tension. Hydrophilicity had a noticeable impact on neurite extension but had little influence on the number of main and secondary branches. In comparison to more hydrophilic scaffolds, a considerably longer total neurite length was found for scaffolds with a surface tension of 40–47 dyn cm^{-1}. This study also showed that neurite elongation is influenced by the distance between fibers. The neurites followed the fibers and steered clear of areas with extremely high fiber densities when the interfiber distance exceeded 15 μm. Neurites moved between the fibers at intervals of less than 15 μm. Therefore, this research came to the conclusion that chemical signals, probably from adjacent neurons, are what trigger the shift in direction [49].

According to the research of Valmikinathan et al., a novel PLGA microsphere-based spiral scaffold was produced with a nanofibrous surface that had increased surface areas, enough mechanical characteristics, and pores to enable the regeneration of nerves. These scaffolds have a uniformly distributed open design that permits enough space for media flow and deeper cell penetration. As demonstrated by in vitro studies utilizing Schwann cells, nanofiber spiral scaffolds encourage more cell adhesion and proliferation than modern tubular scaffolds or nanofiber-based tubular scaffolds [25].

In the Nisbet et al. study, the astrocytic and microglial response was assessed as a result of the implantation of PCL scaffolds produced by electrospinning into the caudate putamen of the adult rat brain. Inflammation decreased to homeostatic levels in a total of 60 days and reached its peak in approximately 4 days in microglia and 7 days in astrocytes. It decreased to homeostatic levels in a total of 60 days. Inflammatory, polymer fiber is unaffected by alignment. Large pores in randomly oriented PCL scaffolds allowed neurite infiltration and growth within the scaffold. When the fibers are partially aligned, neuronal processes cannot penetrate the scaffolds. Instead, at the implant-tissue interface, the PCL fiber grew perpendicular to the alignment direction. That's why vertical contact guidance is provided [50].

In Jurga et al., research, the neurogenic possessions of bioactive scaffolds are produced by cryogelation of extract or gelatin. In their study, they revealed that the pore size is 80−100 microns, which is the optimum pore size for neural tissue replacement. In the rat, these scaffolds were transplanted into organotypic hippocampal slices or brain tissue. According to the results, the scaffolds inhibited the growth of glial scars and did not cause inflammation. However, laminin-rich scaffolds show high neuroregeneration properties [51].

In the study by Li et al., a functionalized collagen scaffold loaded with epidermal growth factor receptor (EGFR), myelin-associated inhibitors, and cetuximab was produced. These scaffolds were developed for NPC implantation. Rat hemisection lesions measuring 4 mm long were implanted with scaffolds containing NPCs expressing green fluorescent protein (GFP) to observe the therapeutic response. Collagen cetuximab (5 mg) decreased the astrocytic differentiation of scaffolds and NPCs, improved axon regeneration, and stimulated neuronal differentiation. Significant functional progress was seen from this. A well-functionalized scaffold was produced to enhance spinal cord injury recovery, in conclusion [52]. Li et al. demonstrated in their work how the growth factor biomaterial structure may provide spatial control of growth factors by leading lineage affiliation of neural stem/progenitor cells (NSPCs) in vivo. The most often employed recombinant proteins were bone morphogenic protein-2 (BMP-2) or platelet-derived growth factor-AA (PDGF-AA), which all include an N-terminal biotin tag and interferon-g (IFN-g). To ascertain NSPC differentiation, these were immobilized on a methacrylamide chitosan (MAC)-based biopolymer and applied to neurons or astrocytes. In chitosan channels, MAC was combined with acrylated

laminin, NSPC, and growth factors before being cross-linked vitro, considerable amounts of growth factors were sustained for 28 days [4].

In the Wang et al. work, scaffolds for the regeneration of peripheral nerves were made using graphene-based nanofibers. The purpose of the investigation was to determine whether the conductive scaffolds that were created might promote the migration of Schwann cells and the impact it may have on nerve regeneration. Reduced graphene oxide was applied to nanofibrous ApF/PLCL (AP/RGO) scaffolds (RGO). Peripheral nerve regeneration was enhanced similarly to autografts when AP/RGO conductive scaffolds were implanted into rat sciatic nerve deficits [53].

In the work of Johnson et al., growth factors and heparin-binding delivery system (HBDS) were produced in fibrin scaffolds. ESNPCs (neuronal progenitor cells produced from embryonic stem cells) were injected into these scaffolds to assess their vitality and neuronal development. In a rat subacute dorsal hemisection lesion SCI model, ESNPCs were implanted. The fibrin scaffold was initially filled with ESNPCs. Second, fibrin containing neurotrophin-3 (NT-3), HBDS, and platelet-derived growth factor (PDGF-AA) surrounds it. Finally, it was enclosed in fibrin scaffolds containing NT-3, PDGF-AA, and no HBDS. The combined use of HBDS-free fibrin scaffold and growth factors boosted the amount of ESNPCs and ESNPCs-derived NeuN-positive neurons in the spinal cords, according to the findings. Four weeks following the transplant, all experimental groups receiving ESNPCs showed an improvement in behavioral function [54].

In the work by Carlson et al., electrospun microfiber polymeric substrates were used to create 3D microtopographic scaffolds. Organotypic hippocampus brain slices were implanted with scaffold-supported neural networks. Multiple neuronal subtypes might be delivered while also increasing survival at the injection site using the transfer of neural networks supported by scaffolds into the striatum of the mouse brain. According to the results, the therapeutic transplant of human neurons demonstrated the potential of 3D microscale biomaterials [55].

12.6 Concluding remarks and future directions

Utilizing the adaptable qualities of biomaterials, neural tissue engineering modifies the nervous system's cells and tissues to regain lost functionality. By transmitting topographical, biochemical, and other signals, implanted biomaterials can affect cell growth and behavior to overcome biological

limitations on brain repair and regeneration. Cues based on biomaterials must be delivered with spatial accuracy and in manageable time periods in order to replicate the natural architecture of the brain. The ability to treat brain damage and illness will be made possible by ongoing improvements in neural tissue engineering techniques based on biomaterials. Complex requirements must be met for functional nerve regeneration. However, experimental research in this area has advanced significantly because to the result of the collaboration of scientists and engineers from many fields.

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