CHAPTER 5

Microfluidic systems for neural tissue engineering

Sumeyye Cesur[1], Songul Ulag[1] and Oguzhan Gunduz[1,2]
[1]Center for Nanotechnology & Biomaterials Application and Research (NBUAM), Marmara University, Istanbul, Turkey; [2]Department of Metallurgical and Materials Engineering, Faculty of Technology, Marmara University, Istanbul, Turkey

5.1 Introduction

One of the most intricate and complex biological structures to form throughout development is the human nervous system [1,2]. The body's motor and sensory functions are impacted when this system, which is the most crucial in the body, is impaired [3]. Anatomically, the central nervous system (CNS) and the peripheral nervous system (PNS) make up the two primary components of the nervous system [4]. It is separated into the somatic nervous system and the autonomic (visceral) nervous system functionally [5]. The spinal cord and brain make up the CNS [6]. In the middle of the vertebral column is the spinal cord. The PNS receives impulses from the CNS, which also houses the visual, auditory, and olfactory systems. The CNS sends and interprets messages. The spinal cord's (spinal nerves) and brain-originating nerves make up the peripheral nervous system (PNS) (cranial nerves). The CNS receives neural signals from the organism's sensory organs and sensory receptors via peripheral nerves. Additionally, it sends neurological signals from the CNS to the body's glands and muscles. The somatic nervous system is made up of the CNS and PNS's neural structures, which process and transmit sensory information (both conscious and unconscious), pain, vision, and unconscious muscle sensation from the body's surface to the head. The autonomic nervous system is responsible for the transmission and processing of sensory inputs from internal organs, including the cardiovascular system and the digestive, respiratory system. Smooth muscle consists of nerve structures responsible for the motor control of the heart muscles and glands of internal organs [5]. There are several types of cellular elements to form brain tissue [7]. Neurons are the basic cellular element of the central nervous system (CNS) and have a highly specialized cell type. It is responsible for initiating, processing, and transmitting the information. In

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addition, their function is influenced by their interaction with glial cells, which shapes the development and survival of synaptic connections and the activity of neuronal systems [8].

5.1.1 Structure of a typical neuron

There are three main regions in a neuron (Fig. 5.1). These are: the cell body (soma), which contains the nucleus and the main cytoplasmic organelles, a single axon, and a variable number of dendrites of different shapes and sizes depending on the neuron type and branching over the cell body. Sensory neural soma clusters are located just outside the neural network. Dendrites are the branched extensions of the nerve cell that spread the electrical impulse from the nerve cells to the cell body or soma of the neuron. The neuron's opposite pole has an axon that is in charge of carrying neural information. This information may be primary information or process information that has already been modified. Neuroglia or glial cells are support cells that hold neurons together, including astrocytes, microglia, and oligodendrocytes in the CNS and Schwann cells and major neuroglial components in the PNS. Astrocytes interact with both neurons and capillaries in the CNS. They support synapses structurally and control the ion and chemical concentrations in extracellular fluid. They also provide nutrients to neurons and try to prevent toxic substances from entering the brain by forming the blood–brain barrier [9]. Microglia are cells called tissue macrophages of the CNS that function as representatives of the immune system in the brain. In the healthy brain, they appear in a long and elaborately branched form with multiple branches and a small cell body. The brain is shielded from microbial invasion by microglia, which also clean and breakdown dead cells [10]. In the CNS, oligodendrocytes wrap myelin

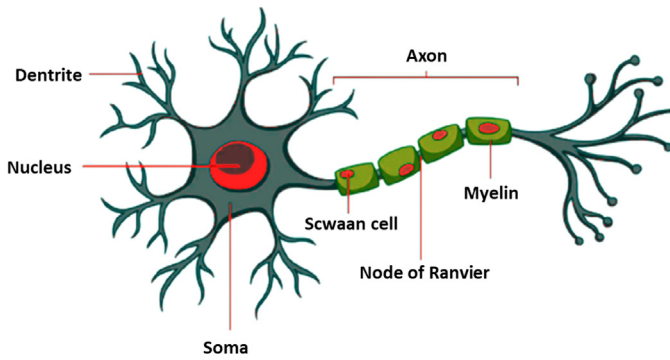

Figure 5.1 Structure of a typical neuron.

sheaths around axons. Numerous oligodendrocytes can myelinate one axon, and one oligodendrocyte can myelinate multiple neurons [11,12]. This is different from PNS. Because the entire Schwann cell surrounds the axon, a single Schwann cell can provide myelin for only one axon. PNS glia consists of Schwann cells and major neuroglial cells surrounding the neuron axon. Myelin in the PNS is produced by Schwann cells. Schwann cells secrete large amounts of extracellular matrix components. Also, Schwann cells, unlike oligodendrocytes, react violently to damage, as do astrocytes [7].

5.1.2 Nervous system injuries

Nervous system injuries, including disruption of axonal bundles, can lead to poor quality of life, lifelong disability, and heavy social and economic burdens. Nerve injuries can affect the spinal cord, brain, and peripheral nerves [13]. Debilitating chronic degenerative diseases and acute traumatic injuries of the nervous system lead to loss of CNS and PNS function. Traumatic injury of the CNS affects millions of people around the world each year. Degenerations in the CNS due to damage to the brain and spinal cord regions cause loss of axons, neuronal cell bodies, and glial support [14,15]. Spinal cord injury (SCI), one of the CNS illnesses, obliterates the neural pathways that link the brain to the rest of the body. It causes paralysis and a loss of feeling below the level of injury [16]. It can come in a variety of shapes and sizes, including trauma from falls, sports-related injuries, violence, and auto accidents [17]. Neurological disorders are also common. Neurodegenerative diseases such as Prion disease, Alzheimer disease, Parkinson disease, Huntington disease are serious health problems that result in cell loss in certain parts of the brain. These diseases are sneaky and progressive, and their prevalence rises as the population ages [18]. PNS injury is the most serious injury. Wallerian degeneration of nerve cell bodies at the distal stump and axon degeneration take place in a small area distal to the proximal stump after a nerve is cut. The cytoskeleton gradually deteriorates and the cell membrane subsequently dissolves [19]. The proximal end of the nerve swells, with only minor damage. After disruption of the cell membrane and skeleton, Schwann cells surrounding axons shed myelin lipids. Schwann cells and macrophages remove the resulting myelin and axon remnants and then produce cytokines that increase axon growth. In order to reach the distal stump, regeneration first starts at the proximal end. New axon shoots

appear in the Ranvier nodes [3]. Because of the presence of excess nerves in the body, the PNS is also prone to traumatic injuries of any kind [20]. The most common types of PNS nerve injuries are traction injury, crush injury, penetrating injury, laceration, ischemia, and heat injury. [21]. Violent penetrating trauma, trauma from motor vehicle accidents, work accidents, and falls are some of the most extensive reasons of traumatic injuries in the PNS. Nerve injuries affect the patient's quality of living because of unbearable neuropathic pain and motor dysfunction. The PNS has the ability to regenerate after injury. Regeneration and repair of both the PNS and CNS remain significant challenges in tissue engineering. Being able to unravel the various mechanisms is important for developing new therapeutic solutions for CNS and PNS injury [22]. There is no clinical treatment modality to improve the repair of CNS injury. Current treatment modalities are not sufficient to restore nerve function in the CNS. Current medical management focuses on orthopedic fixation of an unstable spine, injury prevention, rehabilitation, and preparation of prosthetics. Current treatments for spinal cord regeneration and repair are limited. One of the treatments for spinal cord injury is the administration of methylprednisolone, as acute injury occurs very quickly after the initial injury. It is used to reduce secondary damage processes in excessive doses [23]. However, this therapy has a lot of negative side effects, including sepsis, pneumonia, acute corticosteroid myopathy, wound infection, and gastrointestinal hemorrhage [24]. There is also little progress in neurological recovery. Other treatments for spinal cord injury include surgery to stabilize hypothermia, spinal cord, rehabilitation care, and multisystem medical management. Many clinical treatments have improved. However, therapeutics have not made much progress in the good bridging of injured nerve gaps and full healing of nerve function [25]. In PNS, direct coaptation of cut nerve stumps or the use of nerve grafts is used to repair injured nerves. Autografting, which involves taking a donor nerve from the patient and implanting it into the target place, can frequently provide very effective healing [26]. However, it does have some disadvantages, such as a limited supply of donor nerves, a size mismatch between the donor and recipient nerves, the need for multiple surgeries, and the loss of function at the donor site [27]. Alternatively, allografts and xenografts are used to replace autologous nerve grafts. However, with these treatments, patients are at risk of immune and illness-related complications [28]. Due to the inadequacy of existing treatments, it is necessary to develop new treatment strategies in this area.

5.2 Neural tissue engineering

Tissue engineering uses the principles and techniques of cell biology, materials science, and engineering together. The fact that the nervous system has become easier to understand with the developments here has formed the basis of the area of nervous tissue engineering [29]. Neural tissue engineering provides the best surroundings for the growing and differentiation of nerve cells. It aims to develop new approaches to the treatment of nervous system diseases [30]. Scientists have applied different approaches to improving tissue engineering and neural regeneration by better imitating the in vivo environment. The development of scaffolds that resemble the extracellular matrix (ECM), nano-enabled materials, micro- and nano-topographs, and technologies like three-dimensional (3D) printing, microtechnology, and microfluidics are some of these methods [31–33]. Traditional in vivo cell culture and tissue engineering techniques do not provide an ideal environment for driving or growing neurons. It has shortcomings such as a constrained dispersion of biomolecules and a lack of natural linkages between the ECM and the cells themselves [34,35]. Various approaches have been established to overcome these limitations. These include spheroid- and gel-based systems as well as several 3D-based cell culture techniques. These 3D cell culture systems guarantee a better biomimetic environment and support better differentiation of cells when compared to 2D culture techniques. However, it has limitations in spatiotemporal control of certain cell culture parameters [36]. This has prompted researchers to devise new approaches. With the advent of microfluidic systems, it can control the spatiotemporal distribution of physical and chemical signals at the cellular level. It offers advantages such as analyzing cell differentiation and function using a small number of reagents and fewer cells [37]. Through the simulation of in vivo interactions between the ECM and cells, high-resolution in situ imaging is also made possible [38]. It could be able to get beyond the drawbacks of cell/stem cell culture and tissue engineering methods [39].

5.3 Polymers used for neural tissue engineering

Damage to the nervous system caused by damage or illness can have important or fatal outcomes. The neurological system is difficult to restore because of its intricate physiological makeup [40]. The differentiation of human neural stem cells and the production of nerve channels that can help

in the repair of damaged nerve tissue are applications of synthetic and natural polymers that have shown promise in neural tissue engineering [41,42].

5.3.1 Natural polymers

The use of natural polymers in neural tissue engineering is highly favorable owing to their natural biodegradation kinetics [43], along with properties such as chemically tunable and high biocompatibility. Natural polymers are similar to substances found in the human body and minimize the risks of immunogenic reaction and cytotoxicity at implantation in the body [3,44]. The numerous functions that gelling agents, matrix formers, natural polymers, and drug release regulators play in neural tissue engineering allow them to be easily modified to fit a defect in a difficult and intricate physiological geometry, like the spinal cord [45]. However, poor mechanical properties, thermal sensitivities, and processing difficulties due to their complex chemical structure hinder the effectiveness of natural polymers. Therefore, they are used with synthetic or electroconductive polymers. Collagen, gelatin, chitosan, hyaluronic acid, chitin, elastin, and alginate are natural polymers often employed in neural tissue engineering [40,46].

5.3.2 Synthetic polymers

Synthetic polymers, either biodegradable or not, are employed in neurological applications. While biomaterials containing methacrylate are often not biodegradable [59,60], acetic and glycolic acid polyesters (PLA, PGA) and their copolymer poly(lactic-co-glycolic acid) (PLGA) are employed in PEG-based hydrogels [61,62]. Synthetic materials' chemical and physical characteristics, such as porosity and mechanical strength, can be tailored particularly for a given purpose [6,63]. The integration of neurotrophic factors and the functionalization of synthetic polymers through surface alteration techniques have increased the efficacy of synthetic scaffolds as medication and gene delivery tools to the central nervous system (CNS) [64–66]. Many production procedures, such as freeze-drying, wet spinning, and electrospinning, are suitable with synthetic polymers [67]. Synthetic polymers, on the other hand, have considerable drawbacks. Although these polymers are essentially nontoxic, they may contain toxic residual monomers of incomplete polymerization, disruption crops, and plasticizers. For this reason, before synthetic polymers can be used in the clinic, they must undergo comprehensive testing [40]. Table 5.1 lists the synthetic

Table 5.1 Natural and synthetic polymers in neural tissue engineering.

Polymers	Advantages	Disadvantages	References
Natural polymers			
Collagen	Low antigenicity, adaptability, biocompatibility, lack of cytotoxicity and inflammation, high water absorption capacity, and capacity to modify mechanical and cross-linking properties.	Upon water absorption, there is poor mechanical and structural stability.	[47]
Hyaluronic acid	High water content, strong biocompatibility, dependable degradation products, minimal immunogenicity, viscoelastic qualities, and the capacity to impact wound healing, metastasis, and other processes.	Nonadhesion of cells and water solubility.	[48]
Alginate	Excellent biodegradability, nonantigenic, chelating property, and high biocompatibility.	Lack of specific cell recognition signal, unstable mechanical properties.	[49]
Chitosan	Biodegradability, nontoxicity, biocompatibility, the ability to suppress the growth of bacteria, yeast, and fungi, and the absence of immunogenic characteristics.	Some forms of chitosan can be toxic.	[50,51]
Synthetic polymers			
Polylactic acid (PLA)	Continuous biodegradable fibers with pore diameters between 50 and 350 nm, a high surface-to-volume ratio, and high porosity.	Release of acidic crops during disruption, poor biocompatibility, poor processing capability, and premature deterioration of mechanical properties during disruption.	[52,53]

Continued

Table 5.1 Natural and synthetic polymers in neural tissue engineering.—cont'd

Polymers	Advantages	Disadvantages	References
Poly-lactide-co-glycolic acid (PLGA)	Film-forming capacity, nontoxicity, and biodegradability.	When exposed to stress for an extended period of time, plastic deformation and failure, release of acidic products on decomposition.	[54]
Polycaprolactone (PCL)	High elasticity, good mechanical qualities, minimal toxicity, biodegradability, and a gradual degradation profile.	Cytotoxic effects on the use of organic solvents.	[55]
Polyethylene glycol (PEG)	Highly biocompatible, biodegradable, nonimmunogenic, neuroprotective.	Highly hydrophilic.	[56]
Polyaniline (PANI)	Increased neurite outgrowth, conductive, flexibility, and biocompatibility.	Chronic inflammation and inability to break down.	[57]
Polypyrrole (PPY)	Rigidity, strong biocompatibility and cell adhesion qualities, and lack of toxicity, allergy risk, mutagenesis potential, and hemolytic potential.	Poor processing stability, nonbiodegradable, and insoluble.	[58]
Poly (2-hydroxyethyl methacrylate) pHEMA	Extremely hydrophilic, biocompatibility, and polymerizable at low warmths (between −20°C and +10 °C).	Not biodegradable.	[40]

polymers used in brain tissue engineering. In neural tissue engineering, PLA, PGA, and their copolymer, PLGA, have been used as biomaterials for a range of biological purposes. PGA and PLA polymers are biodegradable and can be hydrolyzed or absorbed in vivo [68]. Both PLA and PGA have previously been used as graft material for wound healing and as a resorbable suture material [69]. Therefore, they are the first tested biopolymers used as a neural tissue for regeneration studies. When combined with natural polymers or covered with cells, biodegradable polymers [70] such as poly(caprolactone) (PCL) can accelerate the regeneration of nerve fibers, enhance cell adhesion properties, or support the directional growth of regenerated axons in peripheral nerves. PEG is widely used in nerve regeneration applications. It increases neuronal proliferation and differentiation, and it has a lot of promise for treating CNS ailments.

5.4 Methods used in neural tissue engineering

To better simulate the in vivo environment and enhance brain regeneration and tissue replacement, scientists have employed a range of techniques. Examples of these methods include scaffolds that mimic the extracellular matrix (ECM), nano-enabled materials, micro and nano-topographs, and technologies like microtechnology, three-dimensional (3D) printing, and microfluidics [31].

5.4.1 Microfluidic systems

Nanotechnology has been applied to various research areas in neuroscience [71]. The application areas include nanofiber scaffolds, nanoparticles that can carry molecules across the blood–brain barrier, nanowires, nanoneedles, and nanotubes used as cellular electrodes [72]. Recently, a microflow system has been developed in nervous system study that enables selected measurements such as axonal/dendritic growth guidance, spatially well-defined cell cultures, reduced sample volumes [73,74]. Microfluidic technology has great promise in the field of tissue engineering as well as its significant impact in the area of biomedicine [75]. This technology was developed as a very small-scale instrument consisting of functionalized microchannels ranging in size from tens to hundreds of micrometers (Fig. 5.2). It has been used to culture a variety of cells in a small fluid volume of 10^{-9} to 10^{-18} L under continuous flow conditions to assess the function of cells, tissues, and organs. These devices are used to deliver drug solutions from on-chip reservoirs on neural implants in vivo studies [76].

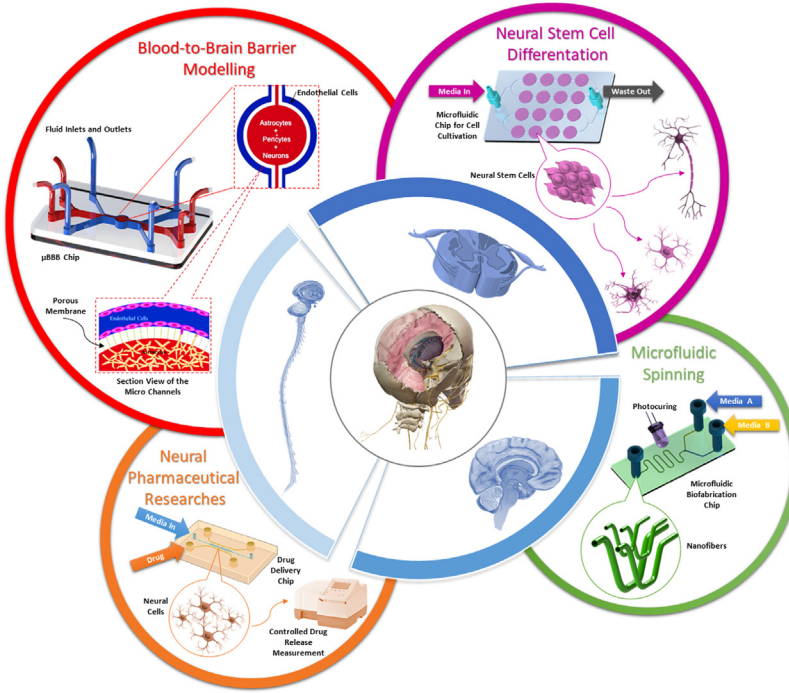

Figure 5.2 Microfluidic application in neural tissue engineering.

However, its most applications have been in vitro studies for protein analysis, DNA sequencing, or the processing and delivery of biochemical molecules for many cell studies [77]. It has also been used for cell sorting, analysis, and flow cytometric cell counting [78]. Currently, microfluidic systems have been fabricated using various methods and materials [79,80]. Among them are the ability to regulate the spatiotemporal distribution of physicochemical signals at the cellular level and the ability to study cell development and function with fewer cells and equipment [81]. In addition, from a cost-saving point of view, the volume of hormones, growth factors, and expensive media is less than that used in conventional culture bottles. This is another advantage of microfluidic experiments. Instead of simultaneously analyzing the reactions of cell populations, studies of individual cells in a controlled microenvironment have the capacity to disclose many aspects of their physiology [74]. Additionally, it offers chances for high-resolution in situ imaging by simulating the in vivo interactions between the ECM and cells. It can overcome the disadvantages of tissue engineering approaches and cell/stem cell culture methods. In this context,

microfluidic technology was developed for cell isolation and differential monitoring, stem cell research, better in vitro disease modeling, and better study of neural development [82,83]. The combination of various advantages of microfluidics can provide solutions for the treatment of additional neurological conditions like Parkinson, Alzheimer, and peripheral or central nervous system injuries or disorders [84].

5.4.1.1 Microfluidic construction

To create the microfluidic chip, the computer software of the design is first used and drawn. The design uses PDMS (polydimethylsiloxane) material to create valves, baffles, and channels. It is the most common microfluidic material used due to its rapid production, high gas permeability, affordable cost, and ease of application. PDMS is a silicone-type elastomer that can then be released from the mold and typically placed on silicone or glass. PDMS allows fluorescence and optical microscopy of confined fluids and cells in the visible spectrum since it is optically transparent down to 280 nm [85]. Its important vantages in cell studies are that it is nontoxic, autoclavable, and gas permeable, allowing the cells in it to breathe. Therefore, if it is not subjected to any pretreatment, it prevents cellular adhesion to its surface [86,87]. Additionally, because it is transparent and shields surface-emitting lasers from the damaging effects of ions in the environment, PDMS is a superb protective covering [88].

5.5 Overview of studies on neural tissue engineering using microfluidic system

Traumatic injuries to the CNS and PNS can both result in irreparable harm and lifelong function loss. By examining the complex dynamics involved in these processes, which can mimic the biological mechanisms of nerve regeneration and degeneration, it may be able to develop novel therapeutics for nerve regeneration and recovery. In vivo animal trauma models have allowed the study of a large number of complicated variables and allowed analysis of behavioral outcomes, making it possible to monitor prognosis and determine functional results for several treatment strategies [36].

Using human ES-derived MNs and iPS-derived ECs, Osaki et al. created 3D brain networks and 3D perfusable vascular networks in macroscale culture and microfluidic devices (iPS-EC). Soft lithography, SU-8-patterned wafers, and a kit of polydimethylsiloxane (PDMS) silicone elastomer were used to create microfluidic devices (Dow Corning,

sylgard184). After being degassed and combined in a weight ratio of 10:1, the silicone elastomer and curing agent were poured over photolithographically printed SU-8 structures and allowed to cure for 6 hours at 80°C. The devices' inlet and outflow ports were made using biopsy punches (1 and 3 mm), which were then autoclaved to sanitize them. Glass coverslips were plasma-bonded to the PDMS layer to form channels roughly 250 m high. The culture medium channels are 1.7 mm wide, whereas the hydrogel's multichannel is 1.25 mm wide. The microchannels were coated with polyDlysine (PDL) for 30 min prior to hydrogel insertion. The findings showed that during perfusion culture, microvascular networks support synaptic connection through direct and indirect communication. Additionally, it has been discovered that MN networks influence the development of vascular networks. These findings showed that bidirectional signaling is essential for healthy function and that the microfluidic modeling of our vascular and neuronal networks can help us understand how these networks interact during crucial developmental processes, the pathogenesis of MND, and mechanobiological phenomena [89].

The creation of potential cell-based treatments for neurodegenerative diseases relies heavily on neural stem cells (NSCs). The mechanistic investigations of NSC differentiation have shown microfluidics to be a potent tool. Wang et al. investigated the relationship between the cell environment existing and C17.2's spontaneous neuronal cell differentiation. Necessary microfluidic design criteria have been proposed to prevent NSCs and unwanted cell phenotype changes in standard culture medium. They designed the microchannels with particular geometric parameters to supply various amount of medium to the cells. The microchannels are made up of PDMS using the soft lithography. SU8 and steel molds were used in this study for devices 50 mm and 250 mm heights and 500 mm, 1 mm, and 2 mm heights, respectively. To prepare the molds for the experiment, they used silicone elastomer base and elastomer curing agent in the ratio of 10:1. They poured this mixture onto the molds and cured at 65°C −75°C. After that microchannels were put on the autoclaved at 121°C for an hour. As a final stage they used oxygen plasma to activate the microchannels and heated for 5−10 min at 65°C −75°C. The medium factor (MF) is the significant parameter to predict the result of C17.2 NSCs in usual culture medium. They found that when the MF is smaller than 8.3×10^4 mm^3/cell.hour, the spontaneous cell differentiation occurred. Then again, when they found the MF equal or larger than 8.3×10^4 mm^3/cell.hour,

minimum spontaneous cell differentiation was observed. According to these results, they found that the MF does not have a powerful correlation with the neurite length [90].

Microfluidic technology was employed in the study by Lee et al. to track the differentiation and migration of neural cells produced from human embryonic stem cells (hESCs). By coculturing hESCs with PA6 stromal cells and subsequently creating neurospheres in suspension culture, isolated neural rosettelike structures were created. Tuj1-positive neural cells were able to enter their microfluidic grooves (microchannels), while nestin-positive neural precursor cells (NPCs) could not. This finding suggests that the ability of neural cells to migrate is influenced by the stage of neuronal differentiation. Axon bundles were seen to develop and expand out into tiny channels. These results demonstrated the potential of microfluidic technology for the study of neurite outgrowth and axon guidance in neural cells derived from human embryonic stem cells [91].

Liu et al. study: a perfused microfluidic device was created to mimic the in vivo intercellular environment. It has been documented how neural stem cells differentiate into neurons. The enhanced amount of III-tubulin production, which was four times higher than that of just cultivating neural stem cells, indicated that neural stem cells cultivated in this microfluidic method demonstrated a high rate of differentiation toward neuron creation. The results of the study demonstrated that multicellular culture systems, such as the perfused microfluidic system reported here, are essential for better simulating the in vivo environment. These factors, which adult neuron cells secrete into the intercellular microenvironment, can stimulate the differentiation of neural stem cells. This study has highlighted the significance of developing multicellular culture systems, such as the perfused microfluidic system, for in vivo studies by demonstrating that some factors secreted by adult neuron cells into the intercellular microenvironment can stimulate the differentiation of neural stem cells [92].

Alginate hydrogel microfibers with diameters of 60∼130 m and 4/8 parallel areas all around them were created in the study by Kitagawa et al. Due to the physical restrictions placed by the relatively hard sections, neuronlike PC12 cells embedded in the parallel region of soft hydrogel matrix multiplied and created linear intercellular networks along the fiber length.

They designed four-layer microfluidic device to fabricate complex hydrogel fibers. The microfluidic devices were made up of poly(methyl methacrylate) (PMMA) plates. A numerical control (NC) machine was used

to form the microchannels array constructs. A drill was used to form the inlet and outlet holes. After that they were thermally bonded at 120° C for 10 minutes and 130°C for 5 minutes. As a final stage, the inlet tubes were glued to the inlet holes. After 14 days of culture, millimeter-long intercellular networks have been successfully produced that structurally mimic the complicated nerve bundles got in vivo [93].

In the study by Hesari et al. a hybrid microfluidic system was created to create a dynamic microenvironment by positioning aligned PDMS microgrooves as physical guiding cues to control the neural development of human-induced pluripotent stem cells on the surface of biodegradable polymers (hiPSCs). Quantitative real-time PCR (qPCR) and immunocytochemistry were used to assess the ability of cultivated hiPSCs in the microfluidic device and other control groups to differentiate into neurons. On rat half-cut spinal cords, functionally differentiated hiPSCs within the hybrid system's scaffolds were also assessed during the acute phase. The state of the implanted cell was assessed using the tissue frozen section, and functional improvement was assessed using the Bresnahan (BBB), Beattie, and Basso locomotor rating scale. Our findings supported the microfluidic device's ability to differentiate hiPSCs into neural cells. In this system, as opposed to those cultivated in other systems such as flat tissue culture sheets and scaffolds lacking fluid channels, the expression of genes unique to neurons was substantially higher. It was thought that the combination of fluid channels with nanofiber scaffolds provides a suitable microenvironment for neural tissue engineering and can be used as a potent tool for in situ monitoring of differentiation potential even though the survival and integration of implanted hiPSCs did not result in a significant functional improvement [94].

In vivo-like extracellular matrix (ECM) can be used to direct the development of neural stem cells (NSCs) in three-dimensional (3D) microenvironments, according to a new technique Han et al. revealed. Poly(dimethylsiloxane) (PDMS) was used to create the microfluidic devices, which had two channels for supplying growth medium on either side and one channel for NSC cultivation in ECMs in the middle of the device. To make the device's disassembly simple, the method of joining the PDMS microchannel component to the flat PDMS membrane has been changed. After autoclaving, the membrane and channel component were both given oxygen plasma treatments, let air in for a while, and then put together. The two pieces formed a reversible bond as a result of this procedure. In order to restore their hydrophobicity, the linked devices were then dried for 12 h at

80°C. In tiny volume ECMs, NSC differentiation was seen and measured. It has been discovered that 3D microenvironments recreated in microfluidic channels dramatically promote the differentiation of NSCs, particularly those that develop into neuronal and oligodendrocytic lineages [95].

In the study by Cheng et al. human placenta-derived multipotent stem cells (PDMCs) were cultured on a microfluidic chip and subjected to chemical and physical stimuli. In comparison to other human stem cells, PDMCs are a recently developed source of stem cells that are simpler to acquire and have fewer ethical concerns. The microfluidic chip created for this study comprises of 1500 mL long and 300 mL wide microchannel connecting 1 mm diameter sample/waste reservoirs to a 5 mm diameter culture chamber. The substance used to create the microchip is PDMS. PDMS was chosen as the chip material due to its high optical transmittance, gas permeability, nontoxicity, and biocompatibility, which are beneficial for cell culture applications. This study showed that PDMC culture on a microfluidic substrate can maintain pluripotency and proliferative capacity while providing an adequate in vitro milieu. On the microfluidic device, PDMCs have successfully undergone chemical and physical-chemical stimulation to develop into neural cells. The findings demonstrated that PDMCs can stimulate physically shear stress to cause increased neuronal cell differentiation [96].

Another study was carried out by Kim et al. they used microfluidic system to observe the neuronal differentiation of NSCs. The ability to self-renew and specialize in many types of nervous system cells is possessed by neural stem cells. Concentrations of soluble biological substances are essential for controlling cell differentiation, migration, proliferation, and death during embryonic development. The ideal culture conditions for the in vitro production of the target cell types were discovered using the microfluidic chip—generated growth factor gradient method. The system used in this study consists of an inlet chamber, an osmotic pump, and a spiral pipe outlet chamber. A concentration gradient was produced at the interface of the two growth factor solutions after the introduction of two solutions with varying growth factor concentrations into the main channel. The two micropipette tips served as an inlet reservoir by being attached to each port and filled with an equal volume of fluid. The microfluidic system's driving force was osmosis, which is created by the concentration distinction between a 0.082M polyethylene glycol and water solution. Flexible polyethylene tubing is wrapped in a small size and utilized as an outlet tank to increase the fluid capacity. The results demonstrated that

NSCs remained healthy throughout cell culture in the microfluidic device and multiplied and differentiated in response to the gradient of growth factor concentration. They also showed that ASCL1 overexpression in NSCs accelerated neuronal development, depending on the concentration gradient of growth factors produced by the microfluidic gradient chip. In the study, they showed how useful the microfluidic chip that produces gradients is for determining the best culturing conditions [97].

In their study, Bae et al. used a PDMS microfluidic system to watch the migration and development of neural cells made from human embryonic stem cells (hESC). HESC and PA6 stromal cells were cocultured, and they separated neural rosettelike structures that later developed into neurospheres in a suspension culture. NPCs and neurospheres were coupled to poly-L-ornithine solution (PLO)/fibronectin (FN)-double-coated microfluidic devices for 5–7 days with the addition of 20 ng/mL BNDF to observe neurite development toward the grooves. The attached NS was positioned within the CO_2 incubator minichamber on an inverted microscope to track neuronal development. For 7–10 days, the medium was placed onto the instrument line and changed every other day. They discovered that the ability of Tuj1-positive neural cells to migrate was reliant on neuronal development and that these cells could fit into the microfluidic grooves. Axon bundles are reportedly generated and dispersed into microchannels as well [98].

In the study of Yang et al. paracrine signaling was used by human mesenchymal stem cells to promote functional neuronal development in 3D extracellular matrices within the microfluidic system of human neural stem cells in a manner that was similar to that found in vivo. hMSCs were created utilizing cationic polymer nanoparticles to express glial cell–derived neurotrophic factor in order to boost paracrine signaling (GDNF). While hMSCs expressing GDNF (GDNF-hMSCs) were cultured in channels situated on each side of the center channel, hNSCs were grown in the 3D ECM hydrogel that was employed to fill the core channels of the microfluidic device. This approach was designed to imitate the paracrine signaling between endogenous hNSCs and genetically modified hMSCs in the brain. In the 3D microfluidic system, coculturing hNSCs with GDNF-hMSCs greatly boosted differentiation into neuronal cells, including dopaminergic neurons, while decreasing hNSC glial differentiation. The electrophysiological characteristics of the neuronal cells produced from hNSCs that differentiated in the presence of GDNF-hMSCs were similar to those of functioning neurons. The enhanced paracrine potential of the GDNF-hMSCs was confirmed in

an animal model of hypoxic-ischemic brain damage. This study showed that paracrine signals from transplanted stem cells can effectively coculture and influence endogenous neuronal activities in vivo using the 3D microfluidic system that was previously reported [99].

In the Lin et al. investigation, a microfluidic chip (μ-CDC) was used to examine how heterogeneous neuronal cells in neurospheres were separated from one another. PDMS was used to create μ-CDC utilizing soft lithography on a glass substrate. Adult mouse brain neural stem cells that had been in floating neurosphere culture for 7 days were resuspended in PBS buffer, placed into a syringe, and injected into the μ-CDD apparatus using a syringe pump at a flow rate of 5 mL/min. A conical tube was used to collect cells from a tube that was attached to the microfluidic channel's end. After the PDMS section of the device was sliced open, cells held in the device were extracted from it using a pipette. The immunofluorescence labeling results showed that nestin- and SOX2-expressing NSCs were separated from other cells near the periphery of the neurosphere. Higher cells were trapped in the NSC markers nestin, SOX2, vimentin, and HDAC1, 2, and 3 as compared to the trapped cells in the device. However, there was less differentiation in p-GSK3β and p-PTEN [100].

The microfluidic device that can create a model of the early developing brain from human embryonic stem cells was created as part of Marc Isaksson's thesis project. In 70% ethanol, the PDMS microfluidic device was sterilized. Cell media with dissolved GSK3i concentrations of 6μM (100%), 3μM (50%), and 0μM were put into four syringes (0%). Syringes and the device's inlets and outlets are connected by long Teflon pipes, as are the waste container and the device's outlets. The microfluidic device was then put in a cell culture dish with only cell media after the syringes were connected. To maintain appropriate contact between the bottom of the Petri dish and the instrument, eight screws and nuts were used to secure the instrument to the instrument holder. The holder/device combination was thereafter put in an incubator set to 37°C. Outside the incubator, in the syringe pumps, are the syringes. The flow rate of the two pumps supplying the gradient generator with cell media was fixed at 40 μL/h. The third pump, which supplied cell media to the culture chamber, was programmed to have a flow rate of 160 μL/h. In order to apply hydrostatic pressure in the culture chamber and cause the bubbles to burst, it was also positioned at a height of 30 cm. Then, all pumps were used continuously for 24 h. The pumps were stopped after 24 h, and the apparatus was put in a new cell culture dish with fixed hESCs on the

bottom. The apparatus was then placed into the incubator. hESCs took 9 days to develop into forebrain, midbrain, and hindbrain cells for the experiment. The findings indicate that the created microfluidic system can be helpful to manufacture many cell types that make up the early brain in a single cell culture. The simulations also demonstrated that it is possible to determine whether the design of the microfluidic system complies with the functional requirements [101].

5.6 Concluding remarks and future directions

The field of neural tissue engineering has difficulties because of the complexity of the nervous system and biological limitations on brain regeneration. The optimal environment for the differentiation and growth of nerve cells is provided via neural tissue engineering. It aims to develop new approaches for the treatment of nervous system diseases. Microfluidic technology is much better than traditional 2D and 3D cell culture techniques and produces good results that are very similar to those obtained naturally. Microfluidics isolates cells and precisely controls their environment. It can offer a fundamental method for examining how various physiological agents, like cytokines, growth factors, and chemokines, interact with specific neurons and glia. It might also make it easier to examine the localized physiology of cell-cell interactions at synapses, axons, or dendrites. The use of diverse biomaterials (natural or synthetic) as a biocompatible scaffold to support nervous system growth is one application of microfluidics in neural tissue engineering. A possible strategy for screening these biomaterials could be microfluidics. Studying combinations of different biomaterials, investigating their comparative efficacy and biological interactions, using a minimal amount of materials are potential advantages of microfluidics. Moreover, assays and long-term cultures are a challenge in neural tissue engineering. Therefore, most biological studies require long-term, high-throughput analyses for precise measurements. In order to quantify their ability for proliferation and differentiation as well as for modeling neurological disorders (Alzheimer, malignancies), drug/biomaterial screening, and high-throughput device use, neural tissue engineering may benefit from applying microfluidics. Microfluidic platforms thus present a novel strategy for experimental study. It may provide crucial new details regarding cellular physiology in both health and disease. It is a good candidate for neuroscience and brain tissue engineering.

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