CHAPTER 2

Introduction to the nervous system from tissue engineering perspective

Yahya Guvenc[1,2] and Can Kivrak[1,2]
[1]Marmara University Neurosurgery Department, Istanbul, Turkey; [2]Marmara University Institute of Neurological Sciences Neurosurgery Department, Istanbul, Turkey

2.1 Introduction

Every year, millions of people are seriously affected by neurological diseases and the damage they cause. Damage to neural tissue is one of the biggest causes of death and permanent disability. In addition to acute traumatic events such as spinal cord injury, stroke, traumatic brain injury, neurodegenerative diseases such as Parkinson and Alzheimer also cause irreversible changes in the nervous system and cause a severe financial burden due to long-term treatments. Due to the physical burden and psychological stress it creates, its effect not only the patients but also their relatives. This proves to be a problem for the wider community. Understanding the neural system's pathophysiology is essential in developing treatments for these pathologies.

The interactions between neurons and neuro-glial (astrocyte, oligodendrocyte, microglia) cells that serve as support tissue are the underlying element of the physiology of nerve tissue. We see the same interaction in the response after the damage. Therefore, all treatment principles aim to regulate the target neuroinflammation, promote axonal growth, and restore myelination.

Currently, no medical interventions can directly repair and restore the neural structure and function in the brain and spinal cord. The biggest problem with neurological injuries is that we have no tools or skills to reconstruct and repair the neural tissue. The complexity of neuroanatomical pathways and networks makes this even more complex. Since nerve cells reach their maximum maturity in the embryonic period, these cells do not divide during the maturation period and this feature is the reason for their insufficient regeneration skills. This low neuroregenerative capacity causes loss of nerve function. Although stem cell–based studies are effective in damaged nerve regeneration, limited integration with neural tissue, cell

Biomaterials for Neural Tissue Engineering
ISBN 978-0-323-90554-1
https://doi.org/10.1016/B978-0-323-90554-1.00010-0

transplantation difficulties, and short-term cell survival after transplantation are the limitations of these studies. The development of 3d scaffolds, which contain progenitor cells, created by neural tissue engineering, creates a support function for cells and is one of the steps to overcome these problems. In this way, neural tissue integration is facilitated, and the necessary physical support for the neural tissue is provided. As understood from this, neuronal regeneration can be made possible by remodeling at the cellular or subcellular levels.

With the advancement of technology in our age, new treatment methods have started to emerge in the field of health. The content and variety of medical and surgical procedures widely used in medicine have also increased with the effect of technology. Organ transplantation, replacement, and repair, tissue engineering, and robotic devices are some options for patients with damaged organs. The list of these treatment options can be increased. However, for any treatment method to be applicable in humans, it must have passed many stages. Many products are eliminated at this stage. Although some products cannot be used, they contain essential knowledge for products to be realized later. The fact that the regeneration of the nervous system is different from other tissues causes the developments in this area to be slow. The inadequacy of traditional methods in repairing nervous tissue has caused studies to focus on this area. Therefore, any research done in this area is valuable.

2.2 Nervous system structure, neurons, neuroglial structure

The nervous system is divided into two parts, the central and peripheral nervous systems. These two centers contain neurons and neuroglia cells. Neuron cells and glial cells originate from the neuroectoderm tissue, which is embryologically differentiated from the ectoderm. Neuroglia cells act as an aid and support to neurons. Although neurons are the primary functioning cells of the nervous system, they cannot divide. Every cell in the body interacts with the surrounding tissue (receiving stimulation from the surrounding tissue, stimulating the surrounding tissue). In neurons, these features are highly developed and specialized. Neuron cell structure consists of soma (stem), dendrite, and axon. The electrical transmission is taken from the dendrites and delivered to the soma, providing a transmission from the body to the environment via axons. Neurons communicate with each other through synapses. When the electrical conduction potential in a synapse

reaches the axon of a neuron, it releases mediator proteins called neurotransmitters from its axon to the synaptic gap. These structures generate electrical impulses in the dendrite of a second neuron. While neuroglia form astrocytes and oligodendrocytes in the central nervous system, Schwann cells form in the peripheral nervous system. Oligodendrocytes form the myelin sheath by wrapping axons in the central nervous system and Schwann cells in the peripheral nervous system. Myelin sheath is known to increase axonal conduction velocity. Unlike neurons, glial cells have cell division abilities.

Peripheral nerve axons are surrounded by a myelin sheath formed by Schwann cells. Peripheral nerves in the peripheral nervous system include motor and sensory axons. All these structures are supported by connective tissue. The endoneurium is the innermost connective tissue covering this structure from the outside. The perineurium surrounds the fascicles formed by the axon bundles, while the fused fascicles are surrounded by the Epineurium. While the epineurium contains supporting vascular structures, the perineurium acts as a blood/nerve barrier (Fig. 2.1).

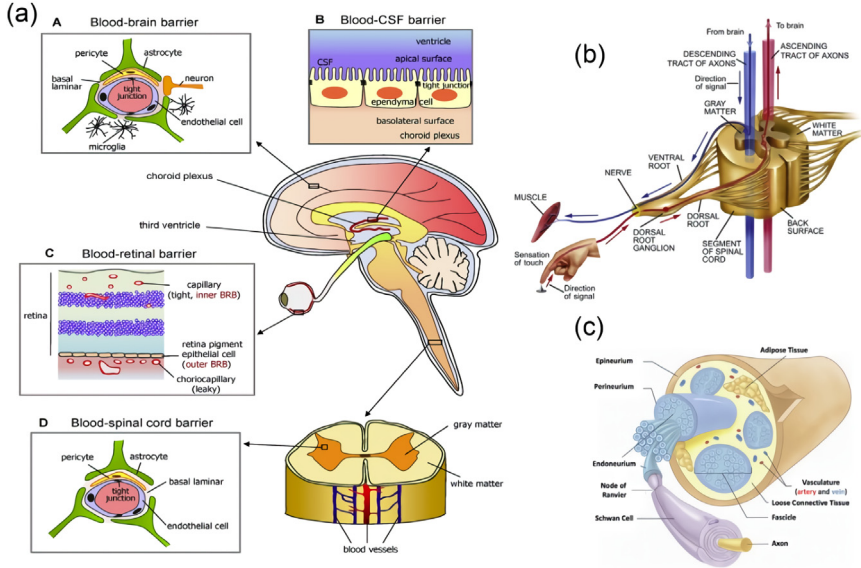

Figure 2.1 (a) Human central nervous system blood barriers. (b) Spinal cord impulse conduction. (c) Peripheral nerve [1].

2.3 Nerve injury and natural regeneration

There are three main types of injury patterns in the peripheral nerve cell: Wallerian degeneration, axonal degeneration, segmental demyelination. After an axonal injury to the nerve, the distal part begins to degenerate due to protease activity. Disruption of the cell membrane structure due to the metabolites released causes the collapse of the cytoskeleton. Macrophages phagocytose it in the medium. The proximal part and body are intact. Cellular swelling occurs in the proximal portion. In the process, first, the excitability decreases, then the axon is disassembled. If the continuity of the nerve sheath is preserved, the nerve regenerates distal to the injury site following degeneration. Changes that spread distally from the lesion area are called Wallerian degeneration. If regeneration does not occur, axon and Schwann cells are replaced by fibroblasts. Although there is a similar process in the central nervous system, information is limited. In the central nervous system, microglia cells phagocytize cellular debris and debris. In segmental demyelination, damage to the myelin sheath or Schwann cells is observed without axonal damage. Since myelin is a renewable structure, remyelination may occur following demyelination. However, there is no reversal in nerve trunk damage. Peripheral nerve injuries are ordered from simple to complex as neuropraxia, axonotmesis, and neurotmesis. The axonal structure is preserved in neuropraxia, and there is local myelin damage. Wallerian degeneration is not observed. Recovery is spontaneous and complete. The axon and myelin sheath are damaged in axonotmesis, while the surrounding connective tissues (endoneurium, perineurium, epineurium) are preserved. There is axonal degeneration. Spontaneous recovery is observed. Neurotmesis is the complete rupture of the anatomical integrity of the nerve. There is a total loss of function. Surgical reattachment should be provided in such injuries. This method can be end-to-end depending on the tension in the nerve tissue, or if it creates tension at the ends, bonding can be achieved by applying a nerve graft between the ends.

2.4 Brain diseases and injury rehabilitation

Neurodegenerative, psychiatric, oncological, neurodevelopmental brain diseases and disorders and brain injury treatments need neurorehabilitation. However, this neurorehabilitation is not sufficient for clinical practice. Therefore, neurotechnologies can support treatment by monitoring and modulating brain activity.

Bioelectronic technologies support us in the understanding of abnormal electrophysiological signals, subtle pathological symptoms, and the modulation of epileptic neuronal activities. The bioelectronic neural treatment creates new opportunities for the long-term, high-quality monitoring of brain signals and using controlled feedback therapies in clinical studies.

Neuroengineering, which includes bioelectric and artificial tissue, is hopeful for various neural diseases, such as Parkinson disease, epilepsy, chronic pain, and neurodegenerative diseases (Fig. 2.2).

2.5 Spinal cord injuries and central nervous system regeneration

Although spinal cord injuries vary in different age groups, they are primarily seen in traffic accidents, falls, violence, and sports injuries. Damage to the neural parenchyma includes disruption of the axonal network, bleeding, and disruption of the glial membrane [3]. Damage to the spinal cord is divided into primary and secondary. This injury model was first proposed by Allen in 1911 [4]. While primary damage is the breakdown of neuron and axonal structures caused by the effect of the mechanical event caused by external factors, secondary damage triggered by this occurs because of molecular and cellular changes such as edema, ischemia, increase in intracellular calcium, increase in excitatory amino acids, the emergence of free radicals. This primary and secondary damage creates permanent changes in the tissue. Some changes occur in the acute, subacute, and chronic periods, resulting in hemorrhage, neural tissue edema, demyelination, axonal and neuronal necrosis, cavity formation, and infarction (Fig. 2.3). The second phase of injury is basically related to the body's biochemical response to trauma. Calcium deposition within cells in tissue causes excitotoxicity in cells, which increases reactive oxygen molecules and glutamate levels. All these events cause degradation in the cell building blocks of neurons. It initiates inflammatory processes such as monocytes, neutrophils, and lymphocytic cells that come to the environment due to bleeding.

Cytokines such as IL-1, IL-6, and TNF alpha produced in the medium exacerbate inflammation in neuronal tissue. Secondary damage occurs within weeks after injury. All these events cause vascular damage to the spinal tissue. The resulting ischemia causes increased cell permeability, triggering apoptotic signals, excitotoxicity, free radical formation, lipid peroxidation, demyelination, Wallerian degeneration, scarring, and finally

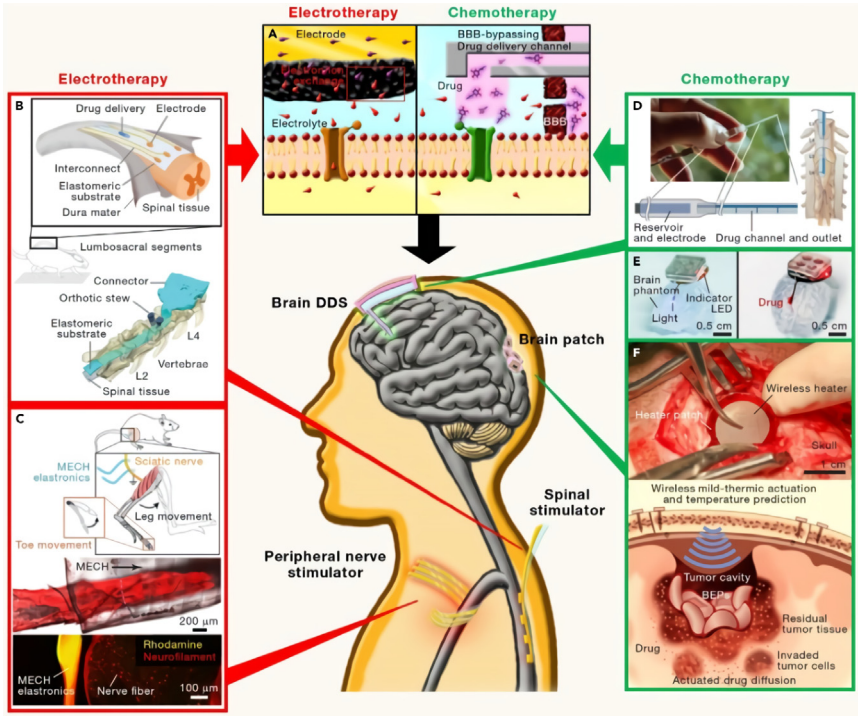

Figure 2.2 Diagrams showing examples of therapeutic applications for soft bioelectronics to the central and peripheral nervous system [2]. (a) Schematic shows materials that can make ion exchange and local drug delivery for electrotherapy and chemotherapy. The soft drug-delivery channel (*gray* layer) is used for bypassing the blood−brain barrier through to the cytoplasm by drugs. It enables to reach the target proteins. (b) The lumbosacral implanted artificial dura is shown schematically. (c) Schematic view and microscopic view of micropatterned electrically conductive hydrogels (MECH) implanted in the sciatic nerve to motorically stimulate mouse limbs. (d) PEDOT: PSS-based bioelectronic devices for recording and modulating electrophysiological and biochemical cell Signals. That schematic shows that the device, which can also release the drugs, is implanted into the spine. (e) Schematic view of brain stimulator that stimulates via chemical or optical. (f) Schematic view of a device that controls from outside via wireless connection, periodically releases drugs for brain tumors. *(b) Reproduced with permission [19]. Copyright 2015, AAAS. (c) Reproduced with permission [20]. (d) Reproduced with permission [21]. Copyright 2015, AAAS. (e) Reproduced with permission [22]. Copyright 2019, National Academy of Sciences. (f) Reproduced with permission [23]. Copyright 2019, Springer Nature.)*

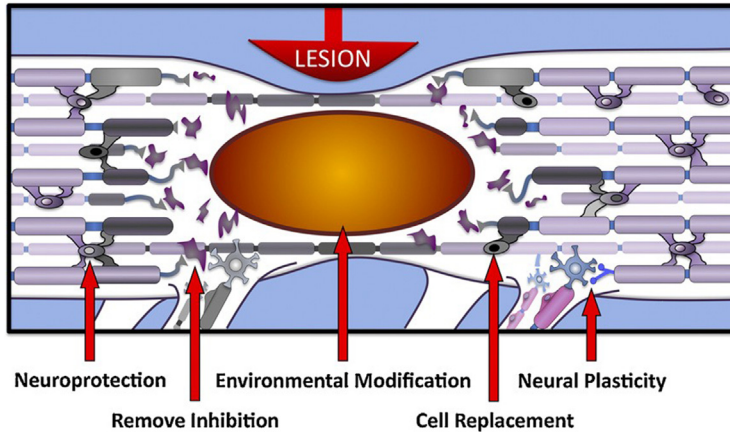

Figure 2.3 Pathophysiology of spinal cord injury. It includes cell loss, necrosis, and glial scarring. These steps are the main targets of bioengineered therapies and are critical for the inflammatory process [5].

cyst formation [6]. Minimizing this secondary damage that starts after an injury is the main goal in clinical treatment.

These injuries can occur due to excessive movements of the spinal column, which protects and supports the spinal cord, in the 3-dimensional plane. Structures forming the spinal column: the vertebrae are the disc structures and spinal ligaments that connect the vertebrae. This combined structure provides a strong support tissue and also allows movement. While flexion, extension, and rotation movements are the basic movements of the spinal column, if the limits of these movements are exceeded, tension and tearing may occur in the neural elements that it protects in the center. Other mechanical factors that cause narrowing of the spinal canal can also cause cord injury. Ligamental hypertrophy, vertebral fracture, or hematoma within the spinal canal may cause spinal cord compression. All these events can cause acute damage as well as chronic sequelae. The main goals of treatment are to restore tissue oxygenation, restore stability, and relieve spinal cord compression. These treatments can be applied medically or surgically.

Many different treatments have been tried from past to present for regeneration in neural injury caused by spinal cord injury. Spontaneous axonal regeneration is observed in the peripheral nervous system in humans, and strong regeneration is not observed in damage to the central nervous system. Until recently, spinal cord regeneration was not understood and

seemed impossible with limited treatment methods. The regrowth and repair of damaged nerve tissues form the complex part of neural regeneration and include elongation of axons, sprouting, or remyelination of nerve cells. With the understanding of the endogenous repair structure of regeneration, electrical stimulation, neurotrophic factors, scar tissue reduction, X-irradiation, neurotrophic factors, grafting, peripheral nerve graft bridges were used, and experimental models were created to stimulate regeneration. Today, although the treatment results are still insufficient, the future is promising with the studies carried out.

2.6 Neural tissue engineering

Neural tissue engineering aims to develop biological structures that mimic natural tissue's structural and physiological nature to maintain, repair, or improve the function of neural tissue or organ affected by disease or injury. While doing this, it uses cell biology, materials science, and engineering principles and techniques together. Neural tissue engineering attempts to provide repair using a biological scaffold, neurotrophic growth factors, and appropriate pluripotent stem cells. Three-dimensional scaffolds and scaffoldlike materials provide the physical support required for successful tissue regeneration. These hyaluronic acid, polymer, and hydrogel-based structures dissolve after completing the integration and support function of neural cells, leaving neural progenitor cells and new cells formed from them in the wound area [7]. While these structures are mainly involved in neuron implantation, they also affect cell behaviors such as neuronal maturation, neuronal differentiation, and stimulating cell behaviors [8].

2.7 Neural tissue scaffolding

Three-dimensional scaffolds are made in two ways, known as conventional methods and techniques using porous polymers that allow cells to attach to the tissue (fiber bonding, electrospinning, solvent casting, membrane lamination, freeze-drying, phase separation, phase emulsion, gas foaming) [9] and additive manufacturing. Additive manufacturing includes 3D bioprinting (extrusion, inkjet, melt stacking modeling, stereolithography) techniques (Fig. 2.4).

While these scaffolds are being formed, they should include some properties such as biocompatibility, biodegradability, conductivity, suitable mechanical properties, and porous interconnection. It should support cell

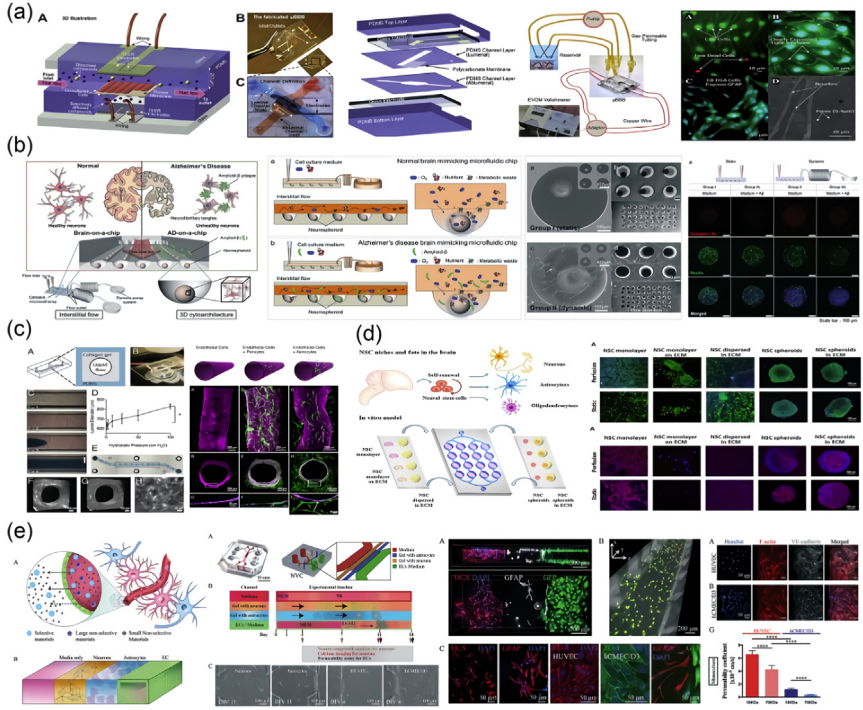

Figure 2.4 These are the in vitro models of a human blood–brain barrier on-chip devices fabricated via soft lithographic fabrication and used in various neuronal applications [1]. (a) Device that is used as a blood–brain barrier [10], (b) a brain mimicking microfluid chip and used in Alzheimer disease models [11], (c) neurovascular inflammation model [12], (d) neural stem cell model [13], and (e) a 3D neurovascular microfluidic system model [14]. *3D*, three-dimensional; *AD*, Alzheimer disease; *BBB*, blood–brain barrier; *μBBB*, microfluidic BBB; *PDMS*, polydimethylsiloxane; *ECM*, extracellular matrix; *HUVEC*, human umbilical vein endothelial cells; *hCMEC/D3*, human cerebral microvascular endothelial cells; *NSC*, neural stem cell.

adhesion, proliferation, and differentiation to mimic natural tissue's extracellular matrix. It should allow the spatial distribution of cells, the exchange of nutrients and waste materials [15].

Natural polymers are advantageous in neural tissue engineering because of their high biocompatibility. Collagen, chitin, chitosan, gelatin, hyaluronic acid, elastin, and alginate are used as natural polymers. It minimizes the risk of developing cytotoxicity and immunogenic reaction [16]. Due to the mixed chemical structure of natural polymers, they have weak mechanical properties, thermal sensitivities, and processing difficulties. Because

of these properties, it is necessary to use synthetic or electroconductive polymers together. Making synthetic polymers more functional by surface modification techniques and using them together with neurotrophic factors are their advantages over natural polymers. However, some situations limit the use of synthetic polymers. Although these polymers are not toxic on their own, disrupting the polymerization process, degradation byproducts, and residual monomers of plasticizers can be harmful. Therefore, it needs extensive testing before clinical application [17].

With tissue engineering, effective treatments for spinal cord injury have developed to the level of tissue reconstruction by reducing the formation of glial scars. The polymer scaffold engineering provides controlled release of drugs targeting the many mechanisms of spinal cord injury.

In conclusion, neuroengineering is constantly evolving according to technology. One of the goals of neuroengineering is to try to restore the structurally and physiologically natural structure of neuron cells that have undergone structural disorders. For this purpose, a scientist produces biomaterials parallel to the developing technology (Fig. 2.5). Effective treatment for spinal cord injury (SCI) would solve glial scars to promote axonal regeneration and reduce secondary effects including inflammation, apoptosis, and necrosis. Three-dimensional (3D) scaffolds provide a platform that affects primary and secondary spinal cord injury (SCI) mechanisms. The other target of neuroengineering provides computational models and machine learning techniques for understanding neural plasticity

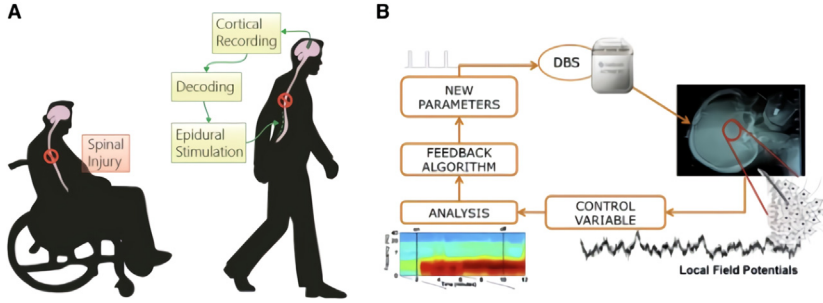

Figure 2.5 Invasive brain-machine interface applications. (a) The figure shows spinal cord injury and the transfer of motor response by voluntary motor stimulation from the upper cortical segment, which is transmitted after the stimulus and bypasses the injury segment. (b) Brain stimulation based on an electrophysiological bioconstructor of subconscious BMI; adaptive deep brain stimulation controlled by local field potentials to treat Parkinson disease. This can't be controlled [18].

processes and motor recovery pathways. Therefore, neuroengineering will allow us to administer customized rehabilitation treatment in the future and will allow us to move toward patient-tailored medicine in the field of neurorehabilitation.

Over the past decades, improving patients' neurological recovery occurred after spinal cord injury (SCI) has remained a challenge. Effective treatment for spinal cord injury (SCI) would reduce not only fractured elements and isolate developing local glial scars to promote axonal regeneration but also ameliorate secondary effects including inflammation, apoptosis, and necrosis. Three-dimensional (3D) scaffolds in spinal cord injury (SCI) have remained a challenging platform in which these mechanisms can be addressed in a controlled manner. Polymer scaffolds with favorable biocompatibility and appropriate mechanical properties have been engineered to minimize cicatrization, customize drug release, and ensure an unobstructed space to promote cell growth and differentiation. These properties make polymer scaffolds an essential potential therapeutic platform. This review highlights the recent developments in polymer scaffolds for SCI engineering.

References

[1] L. Papadimitriou, P. Manganas, A. Ranella, E. Stratakis, Biofabrication for neural tissue engineering applications, Mater Today Bio 6 (2020) 100043.
[2] S.-H. Sunwoo, S.I. Han, H. Joo, G.D. Cha, D. Kim, S.H. Choi, et al., Advances in soft bioelectronics for brain research and clinical neuroengineering, Matter 3 (2020) 1923–1947.
[3] A. Anjum, Y. MDi, M. Fauzi Daud, J. Idris, A.M.H. Ng, A. Selvi Naicker, et al., Spinal cord injury: pathophysiology, multimolecular interactions, and underlying recovery mechanisms, Int. J. Mol. Sci. 21 (2020) 7533.
[4] A.R. Allen, Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: a preliminary report, J. Am. Med. Assoc. 57 (1911) 878–880.
[5] J.T. Wilcox, D. Cadotte, M.G. Fehlings, Spinal cord clinical trials and the role for bioengineering, Neurosci. Lett. 519 (2012) 93–102.
[6] M.R. Dimitrijevic, S.M. Danner, W. Mayr, Neurocontrol of movement in humans with spinal cord injury, Artif. Organs 39 (2015) 823–833.
[7] K. Shimba, C.-H. Chang, T. Asahina, F. Moriya, K. Kotani, Y. Jimbo, et al., Functional scaffolding for brain implants: engineered neuronal network by microfabrication and iPSC technology, Front. Neurosci. 13 (2019).
[8] C.C. Gertz, M.K. Leach, L.K. Birrell, D.C. Martin, E.L. Feldman, J.M. Corey, Accelerated neuritogenesis and maturation of primary spinal motor neurons in response to nanofibers, Dev. Neurobiol. 70 (2010) 589–603.
[9] M.B. Murphy, A.G. Mikos, Chapter twenty-two - polymer scaffold fabrication, in: R. Lanza, R. Langer, J. Vacanti (Eds.), Principles of Tissue Engineering, third ed., Academic Press, Burlington, 2007, pp. 309–321.

[10] R. Booth, H. Kim, Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB), Lab Chip 12 (2012) 1784–1792.
[11] J. Park, B.K. Lee, G.S. Jeong, J.K. Hyun, C.J. Lee, S.-H. Lee, Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease, Lab Chip 15 (2015) 141–150.
[12] A. Herland, A.D. van der Meer, E.A. FitzGerald, T.E. Park, J.J. Sleeboom, D.E. Ingber, Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip, PLoS One 11 (2016) e0150360.
[13] Y. Wang, J. Ma, N. Li, L. Wang, L. Shen, Y. Sun, et al., Microfluidic engineering of neural stem cell niches for fate determination, Biomicrofluidics 11 (2017) 014106.
[14] G. Adriani, D. Ma, A. Pavesi, R.D. Kamm, E.L. Goh, A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier, Lab Chip 17 (2017) 448–459.
[15] T. Bedir, S. Ulag, C.B. Ustundag, O. Gunduz, 3D bioprinting applications in neural tissue engineering for spinal cord injury repair, Mater. Sci. Eng. C 110 (2020) 110741.
[16] J. Ai, A. Kiasat-Dolatabadi, S. Ebrahimi-Barough, A. Ai, N. Lotfibakhshaiesh, A. Norouzi-Javidan, et al., Polymeric scaffolds in neural tissue engineering: a review, Arch. Neurosci. 1 (2014) 15–20.
[17] R. Boni, A. Ali, A. Shavandi, A.N. Clarkson, Current and novel polymeric biomaterials for neural tissue engineering, J. Biomed. Sci. 25 (2018) 1–21.
[18] K.A. Moxon, G. Foffani, Brain-machine interfaces beyond neuroprosthetics, Neuron 86 (2015) 55–67.
[19] I.R. Minev, P. Musienko, A. Hirsch, Q. Barraud, N. Wenger, E.M. Moraud, et al., Biomaterials. Electronic dura mater for long-term multimodal neural interfaces, Science 347 (2015) 159–163.
[20] Y. Liu, J. Liu, S. Chen, T. Lei, Y. Kim, S. Niu, et al., Soft and elastic hydrogel-based microelectronics for localized low-voltage neuromodulation, Nat. Biomed. Eng. 3 (2019) 58–68.
[21] A. Jonsson, Z. Song, D. Nilsson, B.A. Meyerson, D.T. Simon, B. Linderoth, et al., Therapy using implanted organic bioelectronics, Sci. Adv. 1 (2015) e1500039.
[22] Y. Zhang, D.C. Castro, Y. Han, Y. Wu, H. Guo, Z. Weng, et al., Battery-free, lightweight, injectable microsystem for in vivo wireless pharmacology and optogenetics, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 21427–21437.
[23] J. Lee, H.R. Cho, G.D. Cha, H. Seo, S. Lee, C.K. Park, et al., Flexible, sticky, and biodegradable wireless device for drug delivery to brain tumors, Nat. Commun. 10 (2019) 5205.