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Report

Bioengineering and biomedical aspects of treating spinal cord injury

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Abstract

This short report summarizes a paper on bioengineering methods that are effectively applied for the treatment of patients with spinal cord injury (SCI). Biomedical methods such as cell transplantation have been studied to date for the treatment of SCI patients, aiming at cellular repair and nerve regeneration in spinal cord lesions. On the other hand, bioengineering methods described in this paper are used to facilitate the function of neural motor circuits for walking. Here, we would like to describe how bioengineering treatment can be successfully applied to human SCI. The findings of our recent studies are also presented as examples of biomedical studies on SCI.

Key words : spinal cord injury, bioengineering, biomedical, epidural electrical stimulation, ependymal cell, intrinsic regeneration ability

Introduction

Spinal cord injury (SCI) is caused by various kinds of trauma, including contusion, crush, and transection. Tissue repair of the spinal cord lesion is poor. Some tissue repair occurs in the lesion ; however, the spinal cord never recovers its original cellular organization. Usually, the base-

ment membrane is formed at the border between the lesion and surrounding intact spinal cord tissues. The formation of the basement membrane is considered to protect the intact spinal cord tissue from secondary degeneration that occurs following primary injury. Usually, extracellular matrices, including collagen fibers, are scattered throughout the area of the lesion that is delimited by the basement membrane. The lesion is never repaired by CNS cells, including astrocytes and oligodendrocytes, but is generally covered by thin connective tissues, finally resulting in cavities of various sizes. Owing to the disruption of axons extending through the white matter of the spinal cord, motor as well as sensory functions become impaired or ceased below the level of the lesion. After SCI, there is no efficient axonal and/or glial cell regeneration in the spinal cord lesion. This means that motor and sensory functions do not recover after SCI. Almost all studies concerning SCI have focused on how axons can efficiently regenerate through the lesion. Transplantation of various sorts of cells has been studied extensively to accelerate axonal regeneration through the lesion. Thus, cellular recovery, including axonal regeneration, following injury has been the primary topic of SCI studies.

On the other hand, bioengineering techniques have been applied for the treatment of SCI. Such bioengineering techniques can enable patients with chronic SCI to walk, without any intervention to promote cellular repair. The concept of bioengineering treatment of SCI is the functional acceleration of motor neurons innervating muscles. The transduction of a patient's intention to walk into electrical signals is another crucial point in bioengineering methods.

In this short report, we describe the difference between bioengineering and biomedical treatments of SCI.

Ⅰ Bioengineering aspect

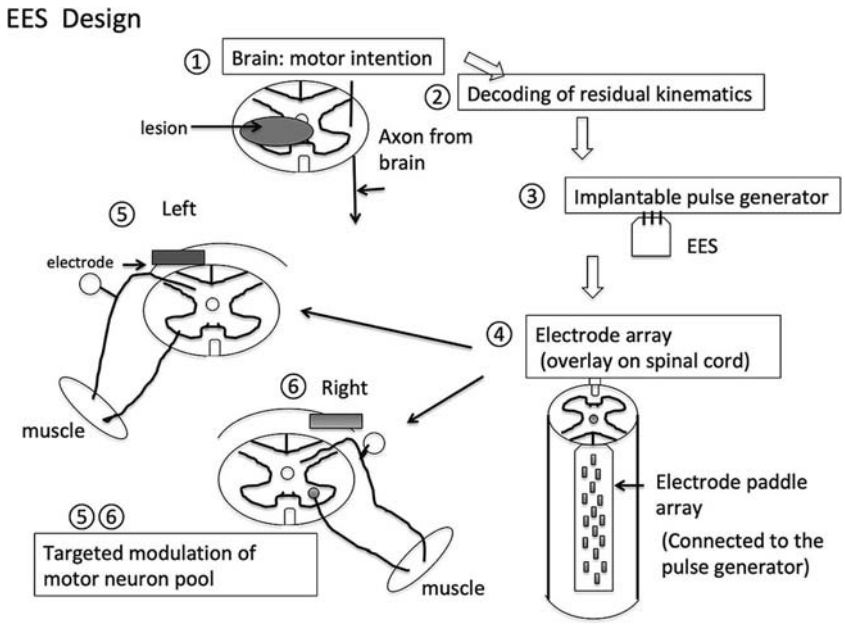
The study by Wagner and colleagues published in Nature (Nature 567 ; 65-71, 2018) is an excellent application of bioengineering for the treatment of SCI. This paper highlights the effect of electrical stimulation of dorsal roots to activate motor neurons in the ventral horn innervated by corresponding dorsal root axons. This paper demonstrates that stimulation of the dorsal roots activates motor neurons in the ventral horn and that motor neuron activation can enable patients to walk by simply supporting the body. The most unique point is that instruments used in this study are all electronic devices. No findings were noted concerning changes in cellular organization during bioengineering treatment. Glial cells and neurons were not referred to in this study. The most notable finding of this study was that patients with chronic SCI can walk on providing simple body support. This study clearly shows that bioengineering techniques can be effectively applied for the treatment of SCI patients.

In the present short report, we would like to describe the experimental setting of this bioengineering paper. This paper shows the marked potential of clinical engineering to treat patients with neural disorders.

(1) The experimental design of bioengineering treatment of human SCI

Signals of a patient's intention to walk are transmitted through axons remaining in the undamaged white matter (Fig. 1, ① and ②). Those residual kinematics are decoded (Fig. 1, ③) and transmitted to an implanted pulse generator, which then sends information to a device called an "epidural electrical stimulation (EES)" system (Fig. 1, ④). This system consists of 16 electrodes arranged as shown in Fig. 1, ④. Electrodes are arranged on a paddle sheet. The paddle carrying electrodes is placed on the surface of a laminectomized spinal cord. Electrodes on the paddle are in contact with dorsal roots with dura mater. This experimental system is called the "16-electrode paddle array" (Fig. 1, ④). Each electrode of EES is connected to a pulse generator. Electrodes are arranged on a paddle so that individual electrodes can be attached to corresponding dorsal roots at the lumbosacral level. These electrodes are controlled to stimulate individual dorsal roots projecting to specific motor neurons in the ventral horn (Fig. 1, ⑤ and ⑥). This paper explains "real-time processing of residual kinematics ensures that targeted EES coincides with movement intent."

Targeted stimulation of dorsal roots excites motor neurons connected with dorsal sensory neurons located in the ventral horn, resulting in a contraction of muscles innervated by corresponding neurons. This is a unique way to induce the contraction of muscle that had been paralyzed due to SCI. They also use devices for decoding residual kinematics conveying the intention of the patient to walk utilizing remaining intact descending tracts in the spinal cord. This information is transmitted to a pulse generator implanted in patients. Information from the pulse generator is transmitted to a 16-electrode paddle array to



Signals of a patient's intention to walk are generated in the brain (①). Such signals are transmitted through axons in the white matter of the spinal cord. Those residual kinematics are decoded (②). Decoded information is transmitted to the implanted pulse generator (③). This generator sends information to a specific device called the "epidural electrical stimulation (EES)" system (④). This system consists of 16 electrodes arranged as shown in ④. Electrodes are attached to a paddle sheet. This experimental system is called "16-electrode paddle array" (④). The paddle carrying electrodes is placed on the spinal cord that had been laminectomized to expose the dorsal roots. Electrodes are in contact with dorsal roots with dura mater. See the text for details.
This picture was drawn based on the paper by Wagner et al., 2018.

Fig. 1

stimulate the posterior roots projecting to specific motor neuron pools. As described above, this system is called EES. The patient is supported by a harness that is connected to a multidirectional gravity assist device to walk.

The motor neurons excited by sensory axons innervating from the dorsal roots induce the contraction of muscles. This means that the paralyzed muscles are activated by electrical stimulation through sensory inputs that are generated by a patient's intention to walk.

Columns of motor neurons innervating muscles involved in walking from the iliopsoas to biceps femoris and gluteus maximus are identified between L1 to S2 levels. The kinds of muscles stimulated by each electrode in the 16-electrode paddle array are examined. Thus, the patient's intention is transmitted to the pulse generator that further sends signals to appropriate electrodes on the paddles. Each phase of walking, i.e., weight acceptance, propulsion and swing, can be well organized on generating information based on the patient's intention. It is surprising that patients with chronic SCI can walk by themselves using EES systems. Although this functional recovery from paralysis is not due to histological repair of the spinal cord lesion, it is remarkable that patients can recover the ability to walk by electrical stimulation.

Bioengineering techniques have a marked potential for application to SCI treatment.

Ⅱ Biomedical aspect

We have been engaged in biomedical treatment of SCI. Here, we would like to describe part of our recent study on cellular reactions in SCI of rats. Cell transplantation has been studied for the treatment of SCI in experimental animals. The premise of cell transplantation is that transplanted cells will survive to be integrated into the host spinal cord tissue and serve as scaffolds for tissue repair, including the outgrowth of regenerating axons in the spinal cord. However, in our studies, bone marrow stromal/mononuclear cells or choroid plexus epithelial cells did not survive long-term, but disappeared from the host spinal cord early after transplantation. Nevertheless, animals showed elevated BBB (Basso, Beattie, Bresnahan) scores for locomotor function, and enhanced tissue repair, including axonal regeneration in the spinal cord lesion. This led to the

hypothesis that transplanted cells might secrete some kinds of neurotrophic factors with beneficial effects on tissue repair, including axonal regeneration, the rescuing of injured axons, and associated glial cell proliferation in the spinal cord lesion. In fact, the conditioned medium of bone marrow stromal cells promoted the improvement of locomotor behaviors and axonal outgrowth through the spinal cord lesion in rats with SCI (Kanekiyo et al., 2017b). This means that the spinal cord has its own intrinsic ability to regenerate mediated by the effect of neurotrophic factors released from bone marrow cells or choroid plexus epithelial cells.

On the other hand, ependymal cells of the spinal cord central canal show extensive proliferation and migration following injury to the spinal cord. Such changes of ependymal cells indicate that they are important for the tissue repair, including axonal outgrowth, of an injured spinal cord, leading to improved locomotor behaviors in experimental animals. This suggests that ependymal cells of the central canal play a primary role in the promotion of the intrinsic ability of the spinal cord to regenerate.

(1) Cell transplantation

Ohta et al. (2004) reported that bone marrow stromal cells (BMSCs) transplanted into the cerebrospinal fluid (CSF) secreted neurotrophic factors, contributing to spinal cord repair and locomotor improvement by stimulating the intrinsic ability of the spinal cord to regenerate. In this study, the spinal cord was injured using a weight-drop at the level of T8–9, and BMSCs were infused into the CSF via the 4th ventricle. Some transplanted BMSCs attached to the spinal cord surface, while a few invaded the lesion. The BBB score was higher and the cavity volume was smaller in rats with transplantation compared with the control. Transplanted BMSCs did not survive long-term to be integrated into the host spinal cord, but gradually decreased in number and disappeared from the spinal cord by 3 weeks after injection. The medium supplemented by CSF harvested from the rats that had received BMSC injection 2 days previously promoted the neurosphere cells to adhere to the culture dish and spread to the periphery. The fact that, although transplanted cells had disappeared within 3 weeks post-transplantation, axonal regeneration and behavioral improvements occurred, indicated that the spinal cord has the autologous ability to regenerate.

Next, the experiment in which BMSCs were injected directly into the lesion in rats with subacute SCI, showed that, although BMSCs had disappeared from the spinal cord lesion by 2 weeks post-transplantation, locomotor improvements and tissue repair, including axonal outgrowth, occurred in the rats (Ide et al., 2010). Almost the same results were obtained in the experiment in which BMSCs were transplanted directly into the spinal cord lesion of rats with chronic SCI (4 weeks after SCI) (Nakano et al., 2013), or bone marrow mononuclear cells (BMNCs) were injected 3 times every week through the CSF in rats with SCI (Kanekiyo et al., 2017a).

Choroid plexus epithelial cells (CPECs) were used as transplants for spinal cord-injured rats. Cultured CPECs were transplanted directly into the contusion-injured spinal cord lesions of rats. Locomotor behaviors evaluated by the BBB score were significantly improved, and numerous axons grew through the spinal cord lesion. Cavity formation was more reduced in cell-transplanted than control spinal cords (Kanekiyo et al., 2016). Since CPECs belong to cells of the central nervous system (CNS), it was expected, prior to experiments that CPECs would survive to be integrated into the spinal cord tissue after transplantation. However, transplanted CPECs only temporarily remained within the lesion, and did not survive long-term. CPECs disappeared from the spinal cord by 2 weeks post-transplantation.

The fact that transplanted BMSCs, BMNCs, and CPECs elicited a marked locomotor improvement and beneficial tissue repair, including axonal regeneration through the spinal cord lesion, suggests that the transplanted cells secreted some neurotrophic and related growth factors in the spinal cord lesion that had effects on axonal outgrowth as well as rescuing injured axons and surrounding glial cells. In fact, the conditioned medium of BMSCs contained various growth factors (Nakano et al., 2010), and promoted locomotor improvement and tissue repair, including axonal regeneration, when injected into the CSF of spinal cord-injured rats (Kanekiyo et al., 2017b).

These findings indicate an important aspect of the properties of the spinal cord : the spinal cord has an intrinsic ability to regenerate following a traumatic injury. The intrinsic ability of the spinal cord to regenerate includes various mechanisms to repair cellular components, including glial cells and neurons, following injury. Among those cellular changes, the most prominent is considered to be ependymal cell proliferation. Ependymal cells of the central canal are considered to

play an important role in tissue repair and axonal outgrowth in the injured spinal cord.

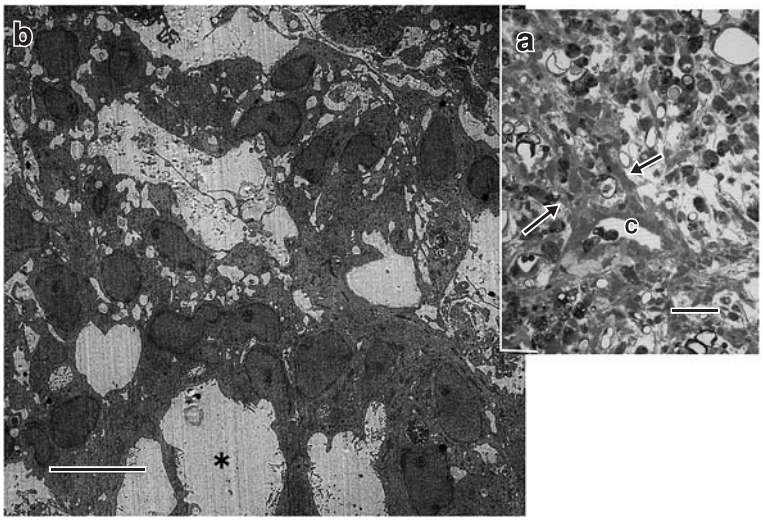
(2) Ependymal cells of the central canal

Ependymal cells line the lumen of the central canal in an epithelial fashion. They are usually arranged in a monolayer without basal laminae on their basal surface, except for those forming the filum terminale (Nakano et al., 2019). It is characteristic for ependymal cells to proliferate extensively in response to traumatic spinal cord injuries. Owing to this characteristic, ependymal cells have been studied as a source of stem cells for multiple neural lineages, including oligodendrocytes, astrocytes, and neurons.

Ependymal cells have been studied from the viewpoint of neural stem cells. Johansson et al. (1999) showed that the mitosis and proliferation of ependymal cells occurred in response to spinal cord injury. DiI-labeled ependymal cells proliferated and migrated into the lesion of spinal cord injury, and some of them exhibited GFAP-immunoreactivity, indicating that ependymal-derived cells differentiate into astrocytes that contribute to glial barrier formation.

The dorsal pole of the central canal contains tanycyte-like cells that produced actively proliferating ependymal cells (Hamilton et al., 2009). This study also showed that ependymal cells of the central canal were bordered by a sub-ependymal layer, and that sub-ependymal cells were latent neural stem cells in the adult spinal cord. Xu et al. (2006) demonstrated that ordinarily quiescent ependymal cells were activated to become nestin+ and undergo an extensive mitosis after SCI. SCI of nestin-promoter green fluorescent protein (GFP) transgenic mice induces the proliferation and migration of ependymal cells. Neurospheres formed from an injured spinal cord contained numerous bFGF+ cells, indicating that cell proliferation is activated by bFGF released from bFGF+ cells. Mothe and Tator (2005) showed that ependymal cells proliferated and migrated from the central canal into the surrounding parenchymal tissue in a minimal spinal cord injury model that preserved the integrity of the central canal. DiI-labeled ependymal cells migrating from the central canal cells were increased by 3 days following injury, and some of them were immunostained for GFAP 14 days post-SCI, indicating that ependymal cells might differentiate into astrocytes. On the other hand, Horky et al. (2006) showed that proliferating ependymal cells differentiated into oligodendrocytes. In their experiment, a cut was made through the dorsal aspect of the cord at T8, and GFP retrovirus was injected at the epicenter of the lesion. Extensive cell proliferation was observed in the central area of the spinal cord, suggesting that those proliferating cells might be derived from ependymal cells of the central canal. GFP-labeled cells were immunostained for NG2, indicating that they belong to the lineage of oligodendrocytes. Barnabe-Heider et al. (2010) conducted a detailed examination of progenitor cells of glial cells in the spinal cord of adult mice. In the intact spinal cord, oligodendrocyte progenitors were the largest population of proliferating cells, while astrocytes and ependymal cells showed limited self-duplication. In contrast, ependymal cells extensively proliferated, generating the largest number of progenitors following injury to the spinal cord. Ependymal cells give rise to progenitors of multiple lineages, serving as neural stem cells in the injured spinal cord. This study suggests that ependymal cells play a major role as progenitors to supply multiple lineages of glial cells to the traumatized spinal cord.

From the viewpoint of spinal cord repair, the proliferation and migration of ependymal cells are considered as cellular reactions involved in forming a cellular framework for tissue repair in the injured spinal cord (Fig. 2). Proliferating ependymal cells differentiate into glial cells, including astrocytes and oligodendrocytes, contributing to the supply of new cells to the injured spinal cord. At the same time, they can be associated with axons to support the outgrowth of regenerating axons and/or rescuing degenerating axons. Thus, ependymal cell proliferation is regarded as the main response of the spinal cord to injury, resulting in the repair of injured spinal cord tissues and the resumption of some motor functions. By observing the proliferation of ependymal cells of the central canal in the early phase following contusion injury, Beattie et al. (1997) thought that those proliferating cells would contribute to the development of cellular trabeculae that provide the framework for cellular infiltration and the regeneration of axons. Cellular trabeculae composed of oligodendrocytes and Schwann cells served as scaffolds for growing axons and astrocytes that are involved in providing the CNS environment. Pioneering work concerning the proliferation of ependymal cells following spinal cord transection was performed in an early electron microscopic study (Matthews et al., 1979). Large primary buds of ependymal cell aggregates were observed extending from the



(a) Proliferation of ependymal cells
Transverse section of the spinal cord 1 week after contusion SCI. Photomicrograph stained by toluidine blue. There is an extensive migration of proliferating ependymal cells (arrows) from the central canal (C). Scale : 30 μm

(b) Electron micrograph showing proliferating ependymal cells
This electron micrograph was taken from a section near that shown in Fig. 1-a. Proliferating ependymal cells can be clearly seen migrating in cell strands, into the spinal cord lesion. They exhibit no special characteristics of differentiated cells. There is a space (asterisk) formed by cells that have microvilli on their apical sides. Proliferating ependymal cells tend to form a central canal-like structure when they are grouped. Scale : 10 μm

Fig. 2

dilated central canal 30-45 days after injury. Those proliferating ependymal cells extended in the form of cellular strands of varying sizes into the tissue surrounding the lesion, and formed miniature cell rosettes similar in structure to the central canal. These cellular reactions of ependymal cells were described as aberrant, abnormal cell proliferation in the spinal cord in that study. Moreno-Manzano et al. (2009) compared the functional properties of ependymal stem/progenitor cells cultured from an injured spinal cord (epSPCi) and those cultured from an intact spinal cord (epSPC) in rats. Neurospheres derived from either epSPCi or epSPC differentiated into oligodendrocytes and motoneurons. They transplanted undifferentiated epSPCi into the contusion-injured spinal cord, leading to significant recovery of motor activity 1 week after injury. This study demonstrated that endogenous cells such as epSPCs could contribute to the locomotor improvement after transplantation for SCI.

Ependymal cells play a major role in the intrinsic activity of the spinal cord to regenerate. It is suggested that the promotion of ependymal cell proliferation and migration will support spinal cord regeneration, including tissue repair and axonal outgrowth, leading to locomotor improvement. Proliferation and migration of ependymal cells following spinal cord injury suggest the presence of mechanisms of intrinsic regeneration of the spinal cord after injury.

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