To celebrate the 20th anniversary of the journal, we have invited several leaders in the field of Tissue Engineering to submit their personal views on past, present and future directions of our discipline. These will appear throughout the anniversary year to celebrate our mutual accomplishments and to point the way toward a bright future for the field.

Antonios G. Mikos, PhD

Peter C. Johnson, MD

John P. Fisher, PhD

John A. Jansen, DDS, PhD

The Editors, Tissue Engineering

Parts A, B and C

Perspectives on Tissue-Engineered Nerve Regeneration for the Treatment of Spinal Cord Injury

Moon Suk Kim, PhD, and Hai Bang Lee, PhD

Over the past few decades, substantial progress has been made to safely improve nerve function in spinal cord injury (SCI) patients through the regeneration of injured nerve tissue. This perspective focuses on an extensive overview of SCI research as well as tissue-engineered nerve regeneration for the treatment of SCI.

SPINAL CORD INJURY (SCI) is caused by trauma, such as that incurred as a result of motor vehicle crashes, falls, physical violence, and sports. As of 2013, 273,000–332,000 individuals in the United States are living with SCI, and an additional 12,000 new cases arise each year.^{1,2} The symptoms of SCI vary greatly, ranging from pain to paralysis. These symptoms are associated with the partial or complete, transient or permanent loss of autonomic, sensory, and motor nerve functions.

Complete SCI may cause discontinuous parenchyma through the lesion, meaning that there is no function in the distal nerves.³ Thus, a complete SCI frequently has a poor prognosis in terms of functional nerve recovery. However, the majority of SCIs are incomplete, causing a partial disruption of nerve tissue in the lesion.⁴ In incomplete SCI, some or all of the motor or sensory functions are retained below the lesion in the spinal cord. Because of recent technical advances, incomplete SCI patients receive better initial surgical care and medical stabilization, leading to longer life expectancies now than a few decades ago.

Although a clear understanding of the underlying mechanisms of human SCI is limited, most injuries are believed to be caused by a fractured vertebra or disk intruding into the spinal canal, following a mechanical insult to the spinal cord paren-

chyma.⁵ In the initial phase of SCI, there is an immediate disruption of the nerve tissue by axon shearing, blood vessel rupture, and necrotic cell death. Subsequently, the invasion of inflammatory and glial cells initiates the secondary phase of nerve cell apoptosis and concomitant demyelination across and beyond the lesion. Consequently, SCI initiates a series of events that lead to the formation of cavities, cysts, and/or glial scars, which act as significant physical and chemical barriers to the regeneration of nerve function. Therefore, the regeneration of damaged nerves spanning the lesion is essential for the development of a definitive post-SCI treatment. Nevertheless, current SCI treatments are aimed at reducing pain and paralysis, rather than promoting the regeneration of nerve function.^{5,6}

Several treatments are currently used to restore the neurological function after SCI. Initial SCI treatments involve surgical decompression of the lesion, immediately followed by the administration of high doses of methylprednisolone and immune modulators, which decrease acute inflammation and further reduce compression on the remaining neurons.⁷ However, several reports have argued against the use of surgical decompression, because the outcome in human trials is not conclusive.⁸ Thus, to date, there is no effective and reliable clinical evidence that surgical intervention is a suitable treatment option.

Over the past few decades, substantial progress has been made to safely improve nerve function in SCI patients through the regeneration of injured nerve tissue. Below, we discuss the clinical research that can potentially be applied to the treatment of SCI patients.

Therapeutic neuroprotective drugs may facilitate the prevention of paralysis and improve the prospect of nerve function recovery after SCI.⁹ In addition, neuroprotective drugs can prevent scar formation if they are administered shortly after the SCI is incurred. Thus, the delivery of a neuroprotective drug shortly after acute SCI might improve future neurological function. Recent research exploring the use of neuroprotective drugs in SCI treatment has yielded valuable preclinical data. However, the majority of these studies are still in the preliminary phase (2 or 3) of clinical assessment.¹⁰

Recent progress has been made toward using cell therapy as a potentially viable therapeutic option for SCI patients. Various cell types, including embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), and more recently, induced pluripotent stem cells (iPSCs), have already provided valuable clinical data regarding nerve regeneration in SCI.¹¹

ESCs can differentiate into several neural cell types, and therefore hold promise as a therapeutic option for SCI patients. The first clinical trials involving the transfer of ESCs into human SCI patients were approved by the U.S. Food and Drug Administration (FDA) in 2009; however, such trials were quickly ceased due to various concerns.¹² Despite the fact that ESCs can develop into benign teratoma tumors, upon the completion of human trials, some companies will provide ESC transplants to treat SCI patients in the coming years.

A recent study reported the use of MSCs to treat SCI patients.¹³ A number of SCI patients with cervical spinal injury experience increased power in their upper limbs and improved performance of daily activities after receiving MSCs. However, MSC therapy exhibited a low remedial value and yielded different results in men and women. Furthermore, MSCs differentiate to form bone or cartilage, not just neurons, across the lesions of SCI patients. In some countries, MSCs obtained from alternative sources, the umbilical cord and adipose tissue, are reportedly being performed in SCI patients, but no conclusive data are currently available.

Human NSCs are also a potential treatment candidate in clinical trials for SCI patients.¹⁴ The SwissMedic regulatory authority have authorized the injection of human NSCs into thoracic SCI patients, and phase 1 or 2 trials are currently underway. Until 2012, there were no safety concerns with regard to NSC transplants. Some SCI patients feel considerable improvement in the sensory function below the level of injury. The clinical trials indicate that transplanted human NSCs might have a positive effect, despite reports that NSCs show minimal therapeutic effects in severe SCI patients.

Recently, progress has been made in using iPSCs as a potentially viable therapeutic option for SCI patients.¹⁵ The use of iPSCs might circumvent the ethical problems associated with the use of embryos and other cells. Clinical-grade iPSCs, which are now in development for SCI patients, are expected to be used in clinical treatments in the near future.

As noted above, most clinical research conducted on SCI patients focuses on treating the lesion directly with therapeutic drugs or cell suspensions in various media. Although recent studies have introduced potentially viable treatments

for SCI patients using drugs or cell suspensions, considerable research is still required to increase the repertoire of successful therapeutic options. The delivery of any therapeutic drug or cell suspension to the injury site poses a challenge, because cell death or drug dissipation occurs on a fast time-scale, and drugs or cells delivered in saline can leak and migrate from the site of transplantation.¹⁶ Furthermore, drug- or cell-based therapies fail to provide topographical guidance of the regenerating neurons, resulting in random growth.

Recently, clinical researchers have explored tissue engineering as a therapeutic option for SCI patients.¹⁷ The scaffold used to engineer tissue must serve several roles in the nerve regeneration process. Ideally, a growth-permissive scaffold that bridges the lesion site might promote neuron extension and, in turn, improve nerve function. The scaffolds also act as a substrate, onto which drugs can be loaded and cells can be seeded before implantation into SCI patients. Scaffolds can act as a local, sustainable, and tunable delivery system for drugs and cells at the lesion site.¹⁸ This is particularly important for therapeutic drugs with *in vivo* half-lives ranging from a few minutes to several days. When a drug is loaded into a scaffold, it prolongs the therapeutic effect from days to weeks. Furthermore, scaffolds can protect the transplanted cells from immune attack, thereby enhancing cell survival. In addition, the scaffold can serve as a barrier to prevent the infiltration of surrounding tissues that could impede the regeneration process. Last, it is easy to match the selected scaffold with the mechanical properties of the nerve tissue modulus in the region of the SCI. Thus, the scaffold can protect or control structural collapse after implantation through controlling the mechanical stability and compatibility of the scaffold.¹⁸

The consensus is that the successful functional recovery of SCI patients cannot simply rely on a single therapeutic treatment. Tissue-engineered nerve regeneration using drug- and cell-loaded scaffolds might have synergistic effects and enhance the regeneration of nerve function after SCI.¹⁷ A key therapeutic strategy is to use neuroprotective drug-loaded scaffolds to inhibit myelin and glial scar formation, or to impart a therapeutic effect on the various cells loaded onto the scaffold. Another approach is to regenerate the damaged neurons using drug- or cell-loaded scaffolds, or to promote the intrinsic growth capacity of neurons across the lesion. In addition, specific scaffold structures can be used to create a growth-permissive topography that guides axonal regeneration across the injury site.

Thus, tissue-engineered nerve regeneration holds the big potential for the treatment of SCI patients, because scaffolds loaded with cells or drugs result in well-integrated, newly differentiated nerve tissue at the lesion. In addition, tissue-engineered nerve regeneration can be useful for treating chronic and acute SCI patients or those with cavities, cysts, and/or glial scars at the injury site.¹⁸

Although there have been significant advances in preclinical studies over the past 20 years, including a number of clinical trials exploring tissue-engineered nerve regeneration, more preclinical experiments are required before advancing to clinical trials.^{17–23} To date, no effective and reliable clinical tissue-engineered nerve regeneration strategies have been available to SCI patients. The rehabilitative capacity of most of the tissue-engineered nerve regeneration strategies has only been examined in animal models.

Although there are few clinical trials pertaining to the treatment of SCI patients and the results obtained so far

are inconclusive, it will be beneficial to continue investigating the long-term adverse clinical effects or complications of tissue-engineered nerve regeneration to improve therapy safety. Currently, a significant challenge for the clinical success of tissue-engineered nerve regeneration is identifying the optimal combination of the scaffold, cells, and/or therapeutic drugs, because certain combinations can produce complicated results. In addition, the generation of reproducible evidence regarding the benefits of using drug- and cell-loaded scaffolds to treat SCI patients also poses a challenge.

To address these challenges, effective translational research, from “bench to bedside,” is required in the near future. Translation research pertaining to tissue-engineered nerve regeneration will require substantial and continuing collaborative research among material, pharmaceutical, biological, and clinical scientists. Because the emerging, clinically relevant information and technology is growing exponentially, future clinical investigations of tissue-engineered nerve regeneration in SCI patients will undoubtedly lead to improved, safer, and more integrated tissue engineering systems that will improve the quality of life of SCI patients.

Acknowledgments

This study was supported by a grant from a Basic Science Research Program (2013R1A2A2A04014200) through the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) and MKE (10038665).

References

1. National Spinal Cord Injury Statistical Center. Spinal cord injury facts and figures at a glance. *J Spinal Cord Med* **36**, 1, 2013.
2. Olby, N., Halling, K.B., and Glick, T.R. Rehabilitation for the neurologic patient. *Vet Clin North Am Small Anim Pract* **35**, 1389, 2005.
3. Helkowski, W.M., Ditunno, J.F., Jr., and Boninger, M. Autonomic dysreflexia: incidence in persons with neurologically complete and incomplete tetraplegia. *J Spinal Cord Med* **26**, 244, 2003.
4. Cholas, R., Hsu, H., and Spector, M. Collagen scaffolds incorporating select therapeutic agents to facilitate a reparative response in a standardized hemisection defect in the rat spinal cord. *Tissue Eng Part A* **18**, 2158, 2012.
5. Burchiel, K.J., and Hsu, F.P. Pain and spasticity after spinal cord injury: mechanisms and treatment. *Spine* **26**, S146, 2001.
6. Sorkin, L.S., and Wallace, M.S. Acute pain mechanisms. *Surg Clin North Am* **79**, 213, 1999.
7. Taghva, A., Hoh, D.J., and Lauryssen, C.L. Advances in the management of spinal cord and spinal column injuries. *Handb Clin Neurol* **109**, 105, 2012.
8. Juknis, N., Cooper, J.M., and Volshteyn, O. The changing landscape of spinal cord injury. *Handb Clin Neurol* **109**, 149, 2012.
9. Blight, A.R., and Zimber, M.P. Acute spinal cord injury: pharmacotherapy and drug development perspectives. *Curr Opin Investig Drugs* **2**, 801, 2001.
10. Priestley, J.V., Michael-Titus, A.T., and Tetzlaff, W. Limiting spinal cord injury by pharmacological intervention. *Handb Clin Neurol* **109**, 463, 2012.
11. Willyard, C. Stem cells: a time to heal. *Nature* **503**, S4, 2013.
12. Chapman, A.R., and Scala, C.C. Evaluating the first-in-human clinical trial of a human embryonic stem cell-based therapy. *Kennedy Inst Ethics J* **22**, 243, 2012.
13. Geffner, L.F., Santacruz, P., Izurieta, M., Flor, L., Maldonado, B., Auad, A.H., Montenegro, X., Gonzalez, R., and Silva, F. Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. *Cell Transplant* **17**, 1277, 2008.
14. Tsukamoto, A., Uchida, N., Capela, A., Gorba, T., and Huhn, S. Clinical translation of human neural stem cells. *Stem Cell Res Ther* **4**, 102, 2013.
15. Nori, S., Okada, Y., Yasuda, A., Tsuji, O., Takahashi, Y., Kobayashi, Y., Fujiyoshi, K., Koike, M., Uchiyama, Y., Ikeda, E., Toyama, Y., Yamanaka, S., Nakamura, M., and Okano, H. Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. *Proc Natl Acad Sci U S A* **108**, 16825, 2011.
16. van den Brand, R., Heutschi, J., Barraud, Q., DiGiovanna, J., Bartholdi, K., Huerlimann, M., Friedli, L., Vollenweider, I., Morad, E.M., Duis, S., Dominici, N., Micera, S., Musienko, P., and Courtine, G. Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* **336**, 1182, 2012.
17. Bell, J.H., and Haycock, J.W. Next generation nerve guides: materials, fabrication, growth factors, and cell deliver. *Tissue Eng Part B* **18**, 116, 2012.
18. Jaklenec, A., Stamp, A., Deweerdt, E., Sherwin, A., and Langer, R. Progress in the tissue engineering and stem cell industry “are we there yet?”. *Tissue Eng Part B* **18**, 155, 2012.
19. Doorn, J., Moll, G., Le Blanc, K., van Blitterswijk, C., and de Boer, J. Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements. *Tissue Eng Part B* **18**, 101, 2012.
20. Caplan, A.I. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* **213**, 341, 2007.
21. Szpalski, C., Barbaro, M., Sagebin, F., and Warren, S.M. Bone tissue engineering: current strategies and techniques-part II: cell types. *Tissue Eng Part B* **18**, 258, 2012.
22. Kang, K.N., Lee, J.Y., Kim, D.Y., Lee, B.N., Ahn, H.H., Lee, B., Khang, G., Park, S.R., Min, B.H., Kim, J.H., Lee, H.B., and Kim, M.S. Regeneration of completely transected spinal cord using scaffold of poly(D,L-lactide-co-glycolide)/small intestinal submucosa seeded with rat bone marrow stem cells. *Tissue Eng Part A* **17**, 2143, 2011.
23. Kang, K.N., Kim, D.Y., Yoon, S.M., Lee, J.Y., Lee, B.N., Kwon, J.S., Seo, H.W., Lee, I.W., Shin, H.C., Kim, Y.M., Kim, H.S., Kim, J.H., Min B.H., Lee, H.B., and Kim, M.S. Tissue engineered regeneration of completely transected spinal cord using human mesenchymal stem cells. *Biomaterials* **33**, 4828, 2012.

Address correspondence to:

Hai Bang Lee, PhD

Department of Molecular Science and Technology

Ajou University

Suwon 443-749

Korea

E-mail: hblee@ajou.ac.kr

Moon Suk Kim, PhD

Department of Molecular Science and Technology

Ajou University

Suwon 443-749

Korea

E-mail: moonskim@ajou.ac.kr

Received: February 3, 2014

Accepted: February 14, 2014

Online Publication Date: March 19, 2014