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Spinal Cord Injury Treatment using a Noble Biocompatible Bridge

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Abstract

The failure of injured axons to regenerate in the mature central nervous system (CNS) has devastating consequences for victims of spinal cord injury (SCI). Traditional strategies to treat spinal cord injured people by using drug therapy and assisting devices that can not help them to recover fully various vital functions of the spinal cord. Many researches have been focused on accomplishing re-growth and reconnection of the severed axons in the injured region. Using cell transplantation to promote neural survival or growth has had modest success in allowing injured neurons to re-grow through the area of the lesion. Strategies for successful regeneration will require tissue engineering approach. In order to persuade sufficient axons to regenerate across the lesion to bring back substantial neurological function, it is necessary to construct an efficient biocompatible bridge (cell-free or implanted with different cell lines as hybrid implant) through the injured area over which axons can grow. Therefore, in this paper, spinal cord and its injury, different strategies to help regeneration of an injured spinal cord are reviewed. In addition, different aspects of designing a biocompatible bridge and its applications and challenges surrounding these issues are also addressed. This knowledge is very important for the development and optimalization of therapies to repair the injured spinal cord.

Keywords: Central nervous system, Spinal cord injury, Biocompatible bridge

Approximately 450,000 people in the United States have sustained traumatic spinal cord injuries (SCI), with more than 11,000 new patients emerging every year. More than 80% of injuries involve males between the ages of 16 and 45. Many millions of populations are also affected worldwide[29,39]. Injury to the spinal cord may involve the destruction of tissue, including the white and gray matter, and blood vessels. Frequent causes of damage are trauma, degenerative processes or stroke, where the amount of tissue damage may increase due to secondary pathophysiologyical changes which is called syringometric cavity. A recent survey in the United States has reported that the leading causes of SCI are motor vehicle accidents (47%), diving injuries (24%), falls (12%), sports and construction works (10%), gun shots and knife injuries (7%)[39,48]. SCI also could occur following surgery where sectioning neural tissue is unavoidable during elective oncological surgery which necessitates removal of a rim of vital tissue from around the lesion[51]. The total direct costs per year for all cases of SCI have been revealed to be $7.736 billion in the United States[16].

Currently, there is no cure for SCI. Clinical research focused on surgical stabilization, medical treatment, and long term rehabilitation of the patient has had little improvement in the overall care of the victims of SCI[33]. However, it is believed that re-growth and reconnection of the axons in the injured region can help to repair SCI completely. Though, transplantation of a variety of cell types, including Schwann cells, olfactory ensheathing glial cells, or neural stem cells, has resulted in axon regeneration, the functional improvement after spinal cord injury is still low[4,15,49].

Biocompatible bridge approach may now be a fascinating new technology that has the potential to revolutionize and regenerate axons with full functional improvement around the wound region. Biodegradable polymers as well as the bridge can simultaneously provide a tissue scaffold, a cell delivery vehicle, and a reservoir for sustained drug delivery[17,18,38]. This integrative approach suggests a possible treatment strategy and may serve as an *in vivo* model for studying optimization of various combinations of treatments. Therefore, an elegant solution may lie in the design of a bio-artificial graft that targets injury mechanisms at the molecular, cellular, and tissue level.

Thus, in this paper, a brief review of the spinal cord

and its injury is represented. In addition, different strategies to help regeneration of an injured spinal cord are included. Using biocompatible bridge as one of the latest and best promising solutions to SCI is further discussed. Different aspects of designing a biocompatible bridge to provide spinal cord regeneration are also highlighted.

Spinal Cord

Neural tissue with supporting blood vessels and connective tissues forms the organs of the nervous system including the brain, the spinal cord, and the receptors in complex sense organs such as the eye, ear and the nerves. Two major anatomical divisions of the nervous system are the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS controls most functions of the body and mind including intelligence, memory, learning and emotion while the PNS (all the neural tissues outside the CNS) is responsible for delivering sensory information to the CNS and carries motor commands to peripheral tissues and systems. The CNS consists of the brain and the spinal cord[36].

The adult spinal cord is about 18 inches long and extends from the base of the brain, down the middle of the back, to about the waist. Figure 1 shows superficial anatomy and orientation of the adult spinal cord. The spinal cord is surrounded by rings of bone called vertebra. These bones constitute the spinal column (back bones). The vertebra is named according to their location. The eight vertebras in the neck are called the cervical vertebra (C-1 to C-8). The twelve vertebras in the chest are called the thoracic vertebra (T1 to T12). The vertebra in the lower back between the thoracic vertebra (where the ribs attach) and the pelvis (hip bone) are the lumbar vertebra (L-1 to L-5). The sacral vertebra runs from the pelvis to the end of the spinal column (S-1 to S-5). The spinal cord conducts sensory and motor impulses to and from the brain and controls many reflexes. The nerves that lie within the spinal cord are called upper motor neurons (UMNs) and their function is to carry the messages back and forth from the brain to the spinal nerves along the spinal tract. The spinal nerves that branch out from the spinal cord to the other parts of the body are called lower motor neurons (LMNs). These spinal nerves exit and enter at each vertebral level and communicate with specific areas of the body. The sensory portions of the LMN carry messages about sensation from the skin and other body parts and organs to the brain. The motor portions of the LMN send messages from the brain to the various parts of the body to initiate actions such as muscle movement. Some of the sensory information is conveyed directly to LMN before it reaches the brain, resulting in involuntary or reflex movements[36].

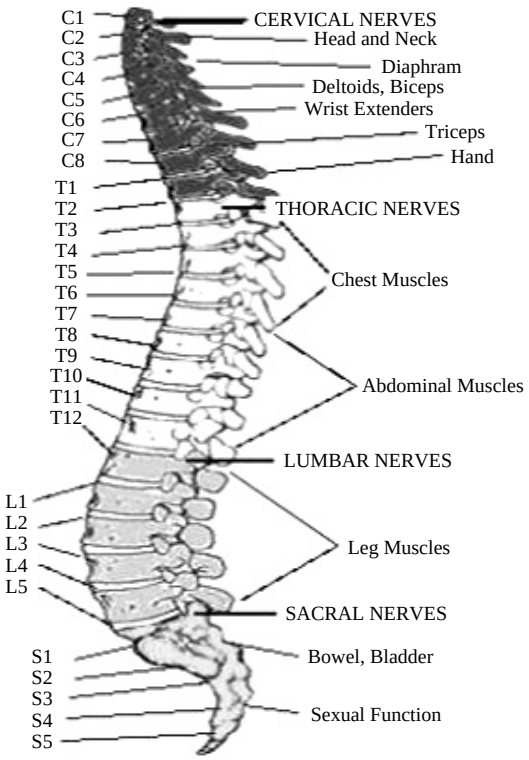

Figure 1. The superficial anatomy and orientation of the adult spinal cord[36].

Injury to the Spinal Cord

Spinal cord injury may be characterized as a result of continuing process of tissue destruction, abortive repair, and wound healing around the injury site. SCI evolves through three phases: (1) acute phase results from initial impact at the moment of injury which leads to immediate mechanical damage of spinal cord and adjacent tissues resulting in massive cell death, (2) secondary phase which starts with a significant shift in the balance of principal electrolytes (Na^+, K^+, Ca^{2+}), proceeds with serious immunological reactions and local inflammatory response, and thereby results to the next phase by expanding the initial core lesion, and (3) chronic phase is the third phase of the SCI in which the complex response to the injury is translated into a quite characteristic cytopathologic pattern[28].

SCI can be divided mainly into incomplete and complete injuries. In an incomplete injury, the spinal cord was not totally damaged or disrupted which means persons with this type injury have some spared sensory or motor function below the level of injury. In a complete injury, nerve damage obstructs every

signal coming from the brain to the body parts below the injury.

The effects of SCI depend on the type of injury and the level of the injury. The level of the injury is very helpful in predicting what parts of the body might be affected by paralysis and loss of function. Cervical (neck) injuries usually result in quadriplegia. Injuries above the C-4 level may require a ventilator for the person to breathe[11]. C-5 injuries often result in shoulder and biceps control, but no control at the wrist or hand. C-6 injuries generally yield wrist control, but no hand function. Individuals with C-7 and T-1 injuries can straighten their arms but still may have dexterity problems with the hand and fingers. Injuries at the thoracic level and below result in paraplegia, with the hands not affected. At T-1 to T-8 there is most often control of the hands, but poor trunk control as the result of lack of abdominal muscle control. Lower T-injuries (T-9 to T-12) allow good trunk control and good abdominal muscle control. Injuries to the five lumbar vertebra (L-1 to L-5) and similarly to the five sacral vertebra (S-1 to S-5) generally result in some loss of functioning in the hips and legs[35].

Besides a loss of sensation or motor functioning, individuals with SCI also experience other changes. For example, they may experience dysfunction of the bowel and bladder[40]. Men's fertility may have been affected as a result of SCI, while women's fertility is generally not affected[7,45]. Very high injuries (C-1, C-2) can result in a loss of many involuntary functions including the ability to breathe, necessitating breathing aids such as mechanical ventilators or diaphragmatic pacemakers[11]. Other effects of SCI may include low blood pressure, inability to regulate blood pressure effectively, reduced control of body temperature, inability to sweat below the level of injury, and chronic pain[1,30]. When the spinal cord is injured, the exchange of information between the brain and other parts of the body is disrupted. In general, the higher in the spinal column the injury occurs, the more dysfunction a person will be experienced[3].

Treatments for the Spinal Cord Injury

Drug Therapy. Drug therapy is the most conventional treatment for SCI[10,42]. Steroid drugs such as methylprednisolone reduce swelling, which is a common cause of secondary damage at the time of injury. Some drugs have the ability to reduce loss of function, although the mechanism is not completely understood[43]. However, the problem with drug therapy is that drugs usually mask the symptoms after the SCI; they do not help in the repair of the damaged tissue or axonal regeneration. Moreover, drug therapy usually results in increasing the drug doses age due to habituation or may lead to undesirable side effects[34].

Technological Assisting Devices. Advanced technology has been used during the last decade to help the people with injured spinal cord. Lighter weight wheelchairs, voice-activated computer and new electrical stimulation devices are the most advanced devices that can help the paralyzed people to perform some of their daily activities[9,13]. New electrical stimulation devices that are implanted in the body have recently been developed that can restore some hand movement. These allow people with SCI to write and feed them[5,14]. Other electrode implants can help better control bladder and bowel function[21,22]. Electrical stimulation devices can also assist with breathing so that some people do not have to be on a ventilator. It is obvious that even the most developed devices can not restore all the biological functions destroyed after SCI. These devices are usually very expensive and not available for all the individuals who suffer from the injury.

Cell Transplantation. Regeneration of injured spinal cord through the axon regrowth has been one of the most promising solutions to this serious problem. One approach to promote axonal regeneration in the injured spinal cord, which has been extensively investigated, is the cellular transplantation e.g. Schwann cells, bone marrow stromal cells, olfactory ensheathing cells, and neural stem cells (NSCs).

Schwann cells have received special interest due to their ability to produce trophic factors, to express a variety of cell adhesion molecules, and to synthesis the extra cellular matrix that is necessary to orient the regenerating axons[19]. Also these cells are available from the host without functional consequences, and the use of immunosuppressant can be eliminated. However, a potential disadvantage of using Schwann cells is their lack of remyelination beyond the injury[4]. Bone marrow stromal (BMS) cells have been grafted *in vivo* into the injured spinal cord by Wu *et al*. (2003)[52]. The grafted cells prompted the regeneration of injured spinal cord by enhancing tissue repair of the lesion, leaving apparently smaller cavities than in controls and resulting in remarkable functional recovery[4,15]. Bartolomei and Greer (2000)[4] reported that ensheathing olfactory cells provide highly favourable substrate for axonal regeneration by secreting extracellular matrix molecules and neutrophic factors.

To date, a number of significant researches on neural stem cells-based therapy to treat spinal cord injury have been presented[6,15,24,49,52]. Radial glial cells are neural stem cells (NSC) that are transiently found in the developing CNS. Koichi H. *et al*. (2005)[32] indicated that acutely transplanted radial glial cells can mig-

rate to form cellular bridges across spinal cord lesions *in vivo* and promote functional recovery following spinal cord injury by protecting against macrophages and secondary damage. However, most of the sources of NSCs are embryonic. Some evidences have suggested that embryonic stem cells are capable of generating any and all cells in the body, under the appropriate conditions. Therefore, they are said to be pluripotent and have unlimited potential as far as growth and differentiation. As these cells grow very fast, we must be careful in fully differentiating them into specialized cells. Otherwise, any remaining embryonic stem cells can exhibit uncontrolled growth and form tumors[2,54]. Thus, transplantation or research conducted with tissues or organs from both embryos and aborted fetuses are still being debated and remain unresolved due to their tumorigenic potential and the ethical concerns of dealing with embryos.

Although, cell transplantation has shown promising results to repair an injured spinal cord, the process of purification and expansion require times and cannot therefore be used in acute injuries. In addition, grafts of cells from other human or animal sources are likely to provoke immune reactions in human patients[18]. Moreover, repair of the injured human spinal cord in many cases require not only neural survival and axonal growth and remyelination but also reconnection across the trauma cavity by means of bridging grafts[38].

Building a Biocompatible Bridge. Recently, building a noble biocompatible bridge at the injured area is a new approach to treat spinal cord injury. From a clinical point of view the limited access to autologous donor material and the immunological problems associated with allograft rejection have prompted a search for artificial biomaterials. Neuroscientists have grafted successfully porous biomatrices into the CNS with the aim of providing the regenerating axons with contact guidance and tunnelled spaces that might orient their growth along the paths of low physical resistance[19,47]. Therefore, at the moment, it seems to be necessary in most cases to construct an artificial biocompatible bridge through the injured area over which axons can grow. Figure 2 represents a biocompatible bridge that can carry axons across a region of damage. It can be divided into three different regions which are the on-ramp, the bridge and the off-ramp.

The on-ramp is the interface between the re-growing axons and the bridge proper. The first problem to overcome the bridge functioning is that the bridge must attract axons into it. This means that cut axons which have retracted for some distance from the injury, must regenerate for a short distance within CNS

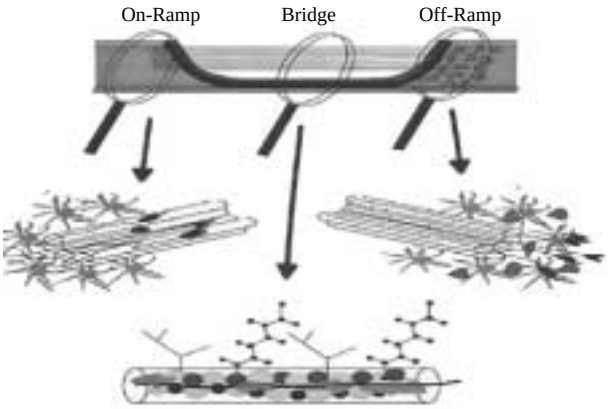

Figure 2. The overall view of the bridge scheme[18].

tissue before they contact the bridge. Then, they must be able to cross from CNS tissue to bridge material. The results of a few researches have shown that some trophic factors e.g. brain-derived neutrophic factor (BDNF) and neutrophin that have chemoattractant properties can provide the conditions for axons to easily enter a bridge implanted after injury[12,20]. This suggests that polymers that release trophic factors and chemoattractants may be advantageous to include within the bridge.

The bridge provides the pathway for re-growth across the lesion area. The bridge can be cell-free or implanted with different cell lines which are called hybrid implant. The bridge must provide surfaces on which the axons can grow, and sufficient physical adhesion to permit that growth. It should not be too adhesive which prevent growth. A major function of bridge surface adhesion molecules is to promote axonal regrowth by activating signalling pathways within the growth cone. The activation of integrin receptors by matrix molecules and cell surface signalling mediated by adhesion molecules both has profound effects that promote axonal growth[41,50]. Thus, the bridge should incorporate molecules that cause the activation of those signalling pathways that are normally activated by the cell adhesion and extracellular matrix molecules.

The off ramp propels the re-growing axons off the bridge and to re-enter uninjured tissue and grow to their final targets. The placement of the bridge graft might result in glial scar formation that may prevent successful implantation. Thus, the problem of the off-ramp is to coax axons from a more permissive environment (the bridge) through an inhibitory boundary (the glial scar) to a less permissive environment (uninjured CNS). Recent work has shown that it is possible, at least *in vitro*, to make axon cross boundaries to

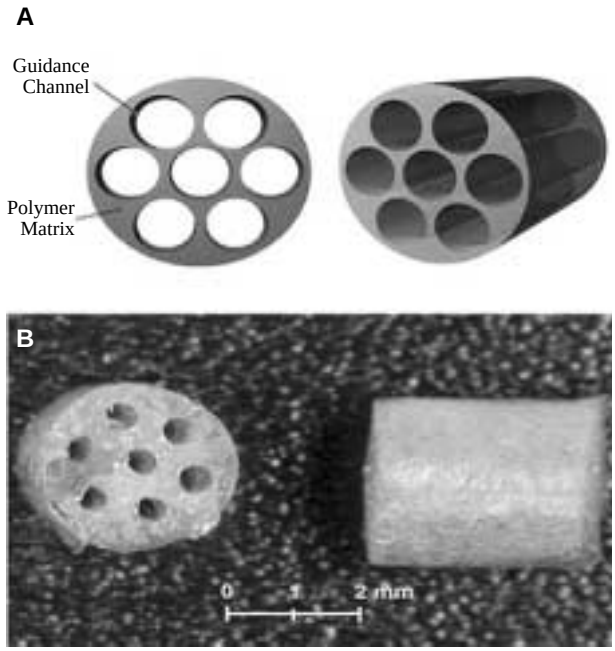

Figure 3. Computer design (A) and photograph (B) of a polymer implant. A, cross sectional (left) and longitudinal (right) views of a polymer implant with parallel, cylindrical guidance channels. B, cross sectional (left) and longitudinal (right) views of a PLGA (85 : 15 lactice/glycolide ratio) implant that is 3 mm in diameter[17].

a less attractive environment by manipulating growth cone signaling mechanisms[8,31]. Growth guidance may also be regulated by levels of intracellular calcium[23]. An additional strategy may be to use intracellular signalling mechanisms to convert a positive chemoattractant signal into a negative repellent signal.

Bridge Design

Each of the regions of a biocompatible bridge has design requirements that are unique to that region and to the axonal population in order to ensure successful regrowth of severed axons. Therefore, bridge structure and materials of its construction are very important for effective design.

The Bridge Microarchitecture. Effective design of various internal bridge microarchitecture may be significant in facilitating robust axon regeneration. A straightforward implant design would be a cylindrical structure, 3 mm in diameter and 5 mm in length with a regular array of cylindrical channels within the polymer matrix. Figure 3 illustrates computer design and photograph of a polymer implant. The hollow internal cylinders would act as guidance channels for axon growth and could be loaded with permissive factors and/or cell types (hybrid strategy) to facilitate

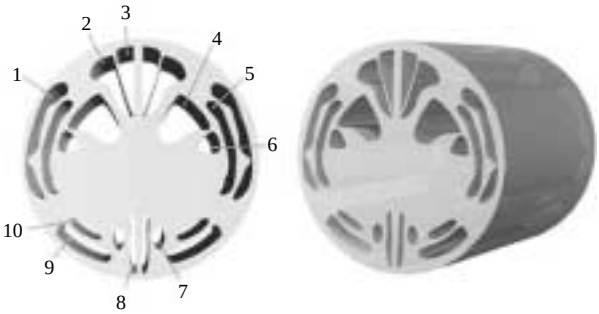

Figure 4. Cross sectional (left) and longitudinal (right) views of an implant with guidance channels corresponding to key spinal cord tracts. 1, spinocerebellar tract; 2, faciculus cuneatus; 3, faciculus gracilis; 4, corticospinal tract; 5, spinothalamic tract; 6, rubrospinal tract; 7, pontine reticulospinal tract; 8, anterior corticospinal tract; 9, vestibulospinal tract; 10, medullary reticulospinal tract.

this process. The optimal diameter of the guidance channel is uncertain, because most biodegradable polymers swell with water to some degree as they hydrolyze. There is likely to be a lower limit of diameter of guidance channel that will support substantial axon growth, depending on the polymer used. The polymer matrix between the channels will serve as a structural support and as a reservoir for the sustained release of therapeutic agents, such as drugs and protein. Although a uniform pattern of cylindrical guidance channels might be a rational starting point, a more creatively patterned internal microarchitecture could be advantageous. For example the channels of implant could be engineered to mimic spinal cord tracts and thereby provide segregation of functional pathway (Figure 4). This method might enhance the specificity of target acquisition and allow for the application of tract-specific growth-enhancing strategies[17,37].

The Bridge Material. A variety of polymers are used to construct the bio-artificial implants. These polymers can be classified as natural polymers, synthetic biodegradable implants, synthetic nonbiodegradable polymers, composite biodegradable implants, and composite conduits with nonbiodegradable scaffold. Various biodegradable natural polymers have been tested for their biocompatibility as potential implants and cell carriers for spinal cord repair. Among them, alginate hydrogel and type I collagen have received particular attention. Synthetic biodegradable implants tested for spinal cord repair include matrigel, fibrin glue and poly (α-hydroxy acids)[26,50]. Synthetic nonbiodegradable polymers used in SCI research are mainly acrylic polymers, including poly N-2-hydroxypropyl-methacrylamide (pHPMA), poly 2-hyroxy-

ethyl methacrylate (pHEMA) and poly pHEMA-co-ethyl methacrylate-methyl methacrylate (pHEMA-co-MMA). Composite biosynthetic conduits combine relatively rigid scaffolds with hydrogel or extracellular matrix molecules. Various cell lines and neutrophic growth factors often complement these types of implants to enhance axonal regeneration. Poly-β-hydroxybutyrate and poly-α-hydroxy acids (mixed with 10% poly-L-lactic acid) are composite biodegradable implants which are used in SCI research. Composite conduits with nonbiodegradable scaffold include conduits with rigid scaffolds filled with hydrogel. The conduits are usually supplemented with different cell lines, embryonic tissue, or neurotrophic factors. Scaffolds tested in SCI experiments are mainly those based on acrylic polymers or polyacrylonitrile/polyvinylchloride copolymer (PAN/PVC)[38].

Recent Studies on Biomaterial Bridge for SCI

Biodegradable hydrogel enhances axonal rewiring and improves performance after spinal cord injury. Gianetti *et al.* (2001)[19] reported the acrylic hydrogels (poly-HEMA) as a cell-free graft in the injured region of the spinal cord of the adult rat. The hydrogel was coated with collagen acting as bio-adhesive substrate. 500-900 μm growth of the central axon was observed which showed a significant continuity in the porous network of the hydrogel implant. Worely *et al.* (2001)[51] demonstrated successful implementation of pHPMA hydrogel containing the cell-adhesive region of fibronectin Arg-Gly-Asp to promote tissue regeneration and support axonal outgrowth in the injured adult rat. Some degree of recovery of motor function was also observed in their experiments. Recently, some studies also demonstrated the effectiveness of hydrogel technology as a clinically feasible delivery system to promote regeneration and enhance functional outcome after spinal cord injury[27,50].

In another study, a multi component Poly (lactic-co-glycolic acid) scaffold with an inner porous layer seeded with neural stem cells (NSCs) have been inserted into a SCI cavity. Numerous regenerating axons were found both in the graft and in the spinal cord caudal to the injury[38,53]. In 2006, Willerth, S. M. *et al.*[53] also optimized fibrin scaffold conditions to promote the differentiation and proliferation of Embryonic Stem (ES) cell in culture, and for use as a platform for neural tissue engineering applications, such as the treatment for spinal cord injury.

Freeze-dried poly (D, L-lactic acid) macroporous guidance scaffolds (foams) with or without brain-derived netrophic factor (BDNF) has been implanted in the transected adult rat thoracic spinal cord[25,41]. Although the results showed that the foam was well tolerated within the injured spinal cord, the overall axonal regeneration response was low[41]. In 2004, 2006, Stokols, S. and Tuszynski[46,47] demonstrated that freeze-dried agarose nerve guidance scaffolds were found to be well integrated with host tissue, individual channels were penetrated by cells, and axons grew through scaffolds in a strikingly linear fashion. Further, incorporating BDNF protein into scaffolds significantly increased the quantity of axons growing into scaffolds[27,46,47].

Next studies carried out by Moore, M. J., *et al.* (2006)[37] showed that multiple-channel, biodegradable scaffolds serve as the basis for a model to investigate simultaneously the effects on axon regeneration of scaffold architecture, transplanted cells, and locally delivered molecular agents. Poly (lactic-*co*-glycolic acid) (PLGA) with copolymer ratio 85 : 15 was used for these experiments.

Future Challenge

Although the promise of biodegradable grafts for the repair of injured spinal cord seems clear, a substantial amount of groundwork must be completed, both at the bench and in animal models, before the implementation of these devices become realistic. A problematic consideration with respect to surgical implementation such as nonpenetrating SCI and iatrogenic transections of the spinal cord need to be performed for successful grafting. This is a major concern, because most cases of SCI do not involve complete transaction at the time of injury. Besides, the optimal timing of implantation and the potential application to both acute and chronic injuries are unknown and need to be established. Moreover, we need to optimize the overall surface area of the bridge that should be sufficient to permit the growth of axons, dictating that it should consist of many small tubes or filaments. The other issue is that only the axonal regeneration after SCI can not result in successful recovery if therapies targeted at the cell body are not administrated concomitantly. These therapeutic goals include the prevention of apoptosis or cells death, growth/growth cone signalling axon guidance, gene expression, stimulation of production of second messengers, and the up-regulation of proteins necessary for neuron outgrowth[17,27,44, 46,47].

Discussion

Soft and elastic biodegradable scaffolds with suitable adhesion molecules and cell lines are likely to represent the first generation of biosynthetic implants for spinal cord repair in humans. Although the results

of using bio-artificial bridge in animal experiments are very encouraging, the translation of experimental therapies to human patients represents a considerable challenge. Successful therapy for SCI requires the fundamental understanding of CNS axon regeneration at both the axon and the cell body levels. Moreover, physical stability, compatibility, immunological reactions, selectivity, absorbability, modifiability and the ability to provide a scaffold for matrix molecules and cellular implants are the most important characteristics which should be considered in designing an efficient bridge. When all these characteristics are achieved, the bridge may serve as the basis for an *in vivo* model used to determine the effects of the structural, cellular, and molecular environments on regeneration of spinal cord axons.

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