

**THE POTENTIAL OF STEM CELLS IN TREATING
SPINAL INJURY AS A RESULT OF PHYSICAL
TRAUMA**

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PASS WITH DISTINCTION

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Abstract

Stem cells are unspecialised cells which have the potential to differentiate into several different kinds of cell, and it is hoped that in the near future we will learn more about controlling the differentiation process to direct the formation of specific cells, tissues and organs.

Trauma-induced spinal cord injuries have a sudden and unexpected impact on people's lives. Depending on the location of the injury, the consequences can be massive such as quadriplegia, or even loss of diaphragm function, meaning a ventilator is required to breathe. We have chosen to look into this area, because "most spinal cord trauma happens to young, healthy individuals¹": people whose lives are likely to be the most affected. This paper is a compilation of our ideas and research into how the properties of stem cells could be harnessed to repair and regenerate damaged tissue in the spinal cord to recover nervous function.

Introduction

An exhibition in Melbourne in 2005² was entitled "Stem Cells: A Biological Repair Kit", and that is in essence what stem cells are. They are able to divide by mitosis and have the potential to become a number of different types of cell. There are various degrees of potency:

- **Totipotent** describes stem cells which can differentiate into any kind of cell within the organism.
- Embryonic stem cells are the stem cells on the inside of the blastocyst (an early stage in embryogenesis) and described as **pluripotent**. They can differentiate to form any of the cells of the developing foetus (but not the cells of the placenta, hence they are not totipotent).
- **Multipotent** stem cells can develop into a number of similar cells, for example different types of blood cells or different cells that make up the nervous system. These are found in adults as well as developing embryos.
- **Oligopotent** cells have a range which is narrower still.
- **Unipotent** stem cells can produce only one type of cell, but can still continue to undergo mitosis³.

Stem cells have been used widely in medicine for decades, for example the transplantation of multipotent cells harvested from the bone marrow which then become blood cells in order to treat diseases such as leukaemia. At present, stem cell research is one of the most exciting branches of medicine, with huge developments being reported on an almost daily basis: "scientists all over the world are engaged in the race to develop stem cell therapies"⁴.

In an organism, stem cells are constantly being used for natural regeneration: most tissues have a supply of unipotent cells which can undergo mitosis to replace necrotic (dead) cells resulting from injury. An example of this is the healing of the skin. However, the Central Nervous System (CNS) is a tissue with a much more limited capacity to heal itself⁵. To see why this is, we must first look at the structure and the cells that make up the CNS.

Histology of the Central Nervous System

The CNS includes the brain and the spinal cord: these are protected by bones (skull and spinal vertebrae) and three membranes (the meninges). The CNS is comprised of two main types of cell: neurons and glia.

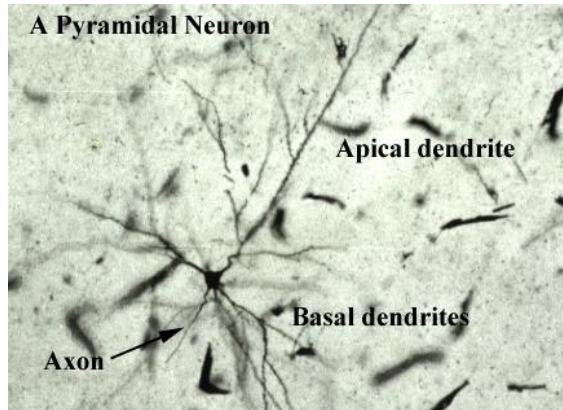


Figure 1: a neural cell body⁶

Neurons transmit electrochemical signals across their plasma membranes, and have a branching structure (as is clearly visible in Figure 1) to connect to adjacent neurons, forming a network between the various sensory receptors, the brain and effectors (which respond to signals from the brain, such as muscle cells). An axon is a long projection that carries the impulses away from the cell body.

Glial cells (comprised a number of different types of cell) surround the neurons and carry out various functions to support them: primarily holding them in place and insulating them from other neurons, supplying them with nutrients and destroying neurons which have died, as well as pathogens.

When damage occurs in the CNS, astrocytes (a type of glial cell) remove the affected neurons by phagocytosis and then divide to fill the space which is left after the removal, forming a glial scar⁷. This has beneficial functions (such as preventing a huge inflammatory response) but the astrocytes in the scar also release chemicals which are thought to inhibit regeneration of neurons and therefore prevent regain of motor function.

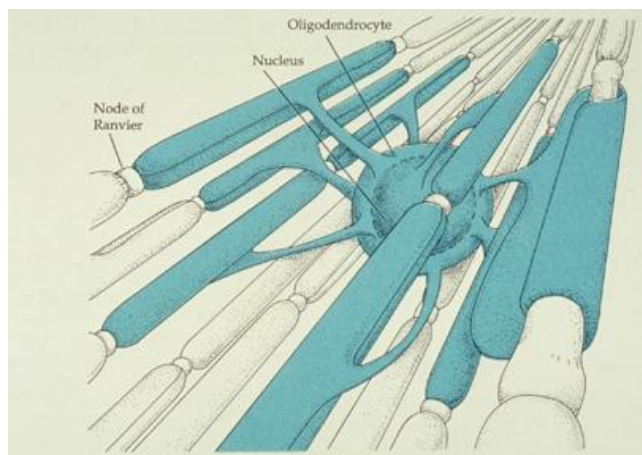


Figure 2: an oligodendrocyte⁸

Another type of glial cell, oligodendrocytes, produces a material called myelin, which acts as a sheath in the CNS, surrounding and insulating the axons of neurons in a similar manner to the plastic coating on a wire (Figure 2).

Often in a spinal injury, the neurons themselves are not completely severed, but they can no longer function due to the necrosis of oligodendrocytes, which are needed for the neurons to remain insulated⁹.

The spinal cord does contain a supply of multipotent stem cells, called neural stem cells (NSCs), but this supply is very limited in the adult spinal cord. After an injury, these stem cells divide and migrate to the affected area, where they differentiate into either the astrocytes, which form the scars, or the oligodendrocytes. Though the astrocytes do have some positive function (as mentioned earlier), they are produced in vast excess of the oligodendrocytes. It is a combination of the inhibitive chemicals produced by the glial scar (inhibiting regeneration of neurons), along with the insufficient number of oligodendrocytes (to myelinate the remaining neurons) which result in the spinal cord's limited ability to heal itself.

The key to reversing the effects of a spinal injury may be in somehow stimulating stem cells to change the ratio and produce more oligodendrocytes and less scar-forming astrocytes.

Discussion

Chemicals Associated with Neurogenesis

Neural progenitor cells (NPs), are oligopotent cells found in the CNS of an adult: as mentioned in the introduction, these are quite specific, with their fate already largely determined¹⁰. It is these that are caused to differentiate into the astrocytes, oligodendrocytes or neurons of the CNS. In a developing embryo, the stem cells are surrounded by a complex extrinsic medium that changes frequently to suit the requirements of constant embryo development. Changes in this environment stimulate or discourage differentiation, so the development of the CNS within an embryo and the chemicals present at different times can be used as a model to study how to regulate differentiate of NPs.

The surrounding extra-cellular matrix acts as a depot for Nerve Growth Factors (NGFs)¹¹ that induce differentiation. These NGFs are released at different stages of neurogenesis – depending on the requirements of the system. Such is the complexity of the cell signalling molecules and their interaction with receptor proteins that little is known about the mechanism triggering the fate of a NSC. We must consider the relationship between the roles and quantities of these chemicals at different stages of neural cell development.

Table 1 summarises the roles of different chemicals in neurogenesis.

Growth Factor	Role
RA	<ul style="list-style-type: none"> • Axon formation • Differentiation of neurons
BMP	<ul style="list-style-type: none"> • Maintain pluripotency • Inhibit differentiation
FGF	<ul style="list-style-type: none"> • Differentiation of neurons
EGF	<ul style="list-style-type: none"> • Cell proliferation • Cell survival
Ezh2	<ul style="list-style-type: none"> • Differentiation of oligodendrocytes
VEGF	<ul style="list-style-type: none"> • Differentiation of astrocytes • Differentiation of neurons

Table 1: The roles of some chemicals involved in neurogenesis

Retinoic Acid (RA) is heavily involved in neural differentiation, specifically axon formation and differentiation of neurons. RA is thought to directly affect the organisation of the posterior hindbrain and the anterior spinal cord¹². RA mediated glial and neural differentiation involves the activation of particular genes within the cell, which then leads to the specialisation of the NP. RA also is closely related to the roles of other molecules such as FGFs and BMP in sensory neurons and motor neurons.

Bone Morphogenetic Protein (BMP) is largely present in the beginning stages of neural development. The main role of BMP is to maintain pluripotency of NSCs and to inhibit neural differentiation, in order to prevent cancerous cells forming. Specifically BMP2, BMP4 and BMP7 play an important role in reducing the rate of neural differentiation¹³.

Fibroblast Growth Factors (FGFs) are a family of many similar NGFs with a wide range of functions, such as neurogenesis and angiogenesis (growth of new blood vessels)¹⁴. Angiogenesis is of relevance because any new tissue will require a supply of blood to function. Also, NPs that show significant expression of genes altered by FGF give rise to neurospheres (clusters of neural cells)¹⁵.

Epidermal Growth Factors (EGFs) bind with the cell plasma membrane protein Epidermal Growth Factor Receptor (EGFR). This connection stimulates intrinsic activity whereby glycolysis and protein synthesis rate increases, which ultimately results in DNA replication and cell proliferation (division)¹⁶. EGFs mainly are used to regulate cell proliferation and aid in the survival of neural cells¹⁷.

Enhancer of zeste homolog 2 (Ezh2) is a polycomb group protein¹⁸ that silences some of the genes present for cell differentiation – thus preventing NSC specialisation into certain cell types and promotes self-renewal of the cell. A study published in 2008¹⁹ shows the expressions of the gene Ezh2 decreased as NSC differentiated into neurons and was completely repressed in astrocytes. However it was shown to be uninhibited in the cells that line and insulate neurons, oligodendrocytes. This research has shown an almost direct correlation between the ratio of oligodendrocytes to astrocytes and expression of Ezh2.

The gene **Vascular Endothelial Growth Factor (VEGF)** has been shown to improve NSC differentiation and survival in mice. When injected into an area close to the spinal cord lesion, the affected NSCs migrate towards the lesion and differentiate to astrocytes and neurons. Although the recovery of the mice was not entirely perfect, the specimens that over expressed VEGF showed significant improvement and longer post operative life span compared to that of the control groups²⁰.

There is still a considerable amount of work required to discover the true nature of these chemicals, and it can't be assumed that their roles in humans will be comparable to that in other organisms such as mice. However, progress is being made and every day more is learnt about these chemicals' complex properties.

Anatomy of the Spinal Cord

Another point to consider is the method of implanting stem cells into the structure of the spinal cord. Our two propositions here are thus:

- the differentiation and growth of an entire section of spinal cord could be achieved *in vitro*, prior to surgical implantation
- the stem cells, after initially dividing, could be injected into the damaged area to grow of their own accord, being stimulated with supplementary NFGs taken by the patient as drugs

To grow the component parts of the spinal cord, it is necessary to first understand the anatomy of the cord itself.

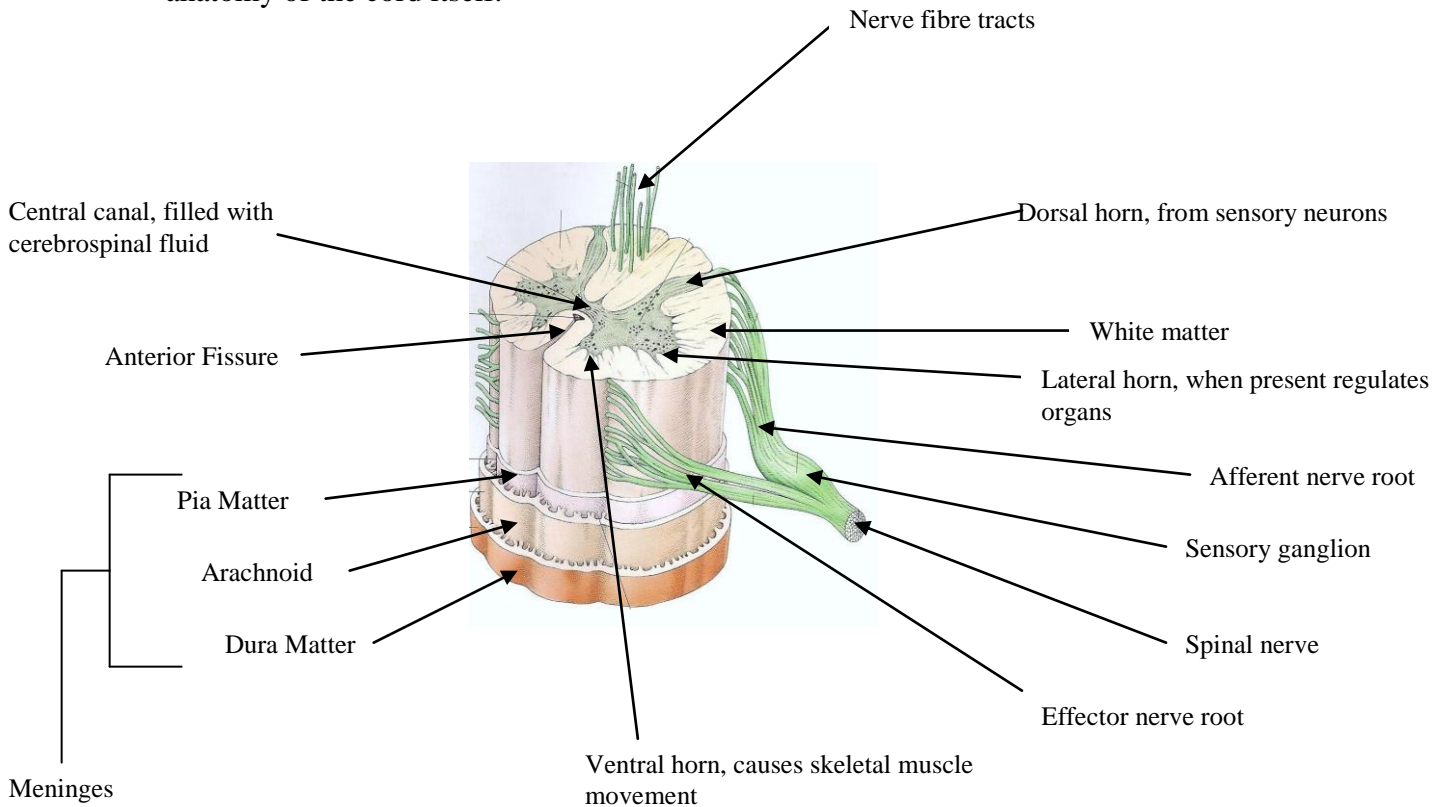


Figure 3: section through the spinal cord²¹

The spinal cord is a complex bundle of neural and glial cells arranged to form the main pathway for neural transmission from the brain to the body (Figure 3). The spinal cord begins at the base of the brain at the *medulla oblongata* and travels through the middle of 4 sets of ring shaped vertebrae, the cervical, thoracic, lumbar and sacral vertebrae. Between each vertebra anterior and posterior roots leave the cord forming spinal nerves, which carry impulses to the respective area/areas which they control. The cord finishes in the *filum terminale*, anchored to the coccyx.

Neural Synapses

Another important aspect of the nervous system is the existence of neural synapses.

Synaptic clefts are the spaces between the axon of one neuron and the cell body of its neighbouring neuron, normally measuring about 20nm across²². The electrochemical signals which travel across the plasma membranes of the neurons cannot cross these junctions, so instead they stimulate the release of a neurotransmitter substance from the presynaptic neuron. This bridges the gap and brings about a change in the postsynaptic neuron, allowing the impulse to continue on its journey (see Figure 4).

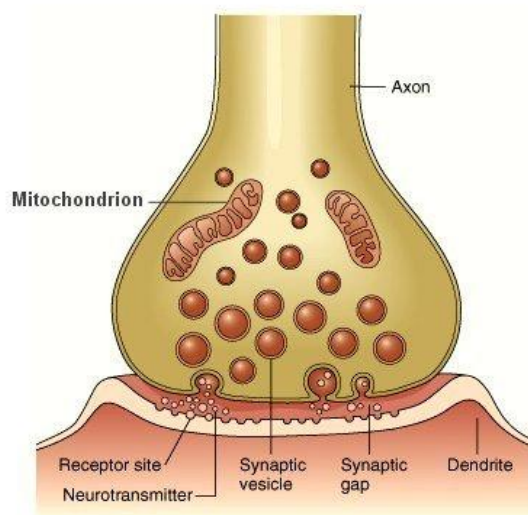


Figure 4: a synapse²³

Evidently, for specific impulses to be sent to specific receptors in order to control bodily function, the synapses must form in exactly the right locations, between the right pre and postsynaptic neurons. Directing the dendrites and axons to undergo synaptogenesis (creation of new synapses) in the correct positions is a difficulty that must be overcome in the regeneration of neural tissue, and this is one of the key challenges our research is focussed on solving.

Method 1

The first solution is growing a new section of the cord *in vitro*. This has huge potential, as the same technology could be used to re-grow not just the neural bundle, but also the branching spinal nerves which would attach to the sympathetic ganglions, parasympathetic ganglions, to the skeletal muscles and the organs.

The NSCs would be harvested and stimulated by the NGFs which cause them to differentiate into neurons and oligodendrocytes (carried out *in vitro*). The neural cells could then be “stacked together” to form a bundle of nerve fibres to form the white matter of the cord allowing vertical transmission between the brain and spinal nerves.

To produce the characteristic shape of the spinal cord, a mesh constructed in the desired shape could be used. This could be constructed from chemicals such as poly[lactic-*co*-glycolic] acid (PLGA), which is used in biodegradable sutures²⁴. This would be broken down over time by the body, leaving just the tissue in the correct shape and position. To form a shape complimentary to that required, the inside surface of this mould would be coated with the NSCs and NPs²⁵. This structure would then be immersed into a solution of an appropriate growth medium (liquid cell culture medium)²⁶ and rotated until on the inside, the cells grow into the shape of the grey matter (similar to the procedure used to produce Claudia Castillo’s new bronchus). The chemicals described earlier could be strategically used at this stage to simulate the environments *in utero* that allow the spinal cord to develop initially by the differentiation of the neurons, oligodendrocytes and astrocytes.



Figure 5: images showing a bioreactor used to grow Claudia Castillo’s new bronchus, similar to that which we would use to grow the section of spinal cord²⁷.

In this biological reactor, the bundles of nerve fibres (all ready grown) must join with the grey matter, to allow vertical transmission between brain and spinal cord. To encourage connections between nerve cells and produce synaptic linkages, the barrier of spatial arrangement and alignment of the dendrites must be overcome. To do, so we again propose two solutions.

The first is to use chemicals like PLGA (described above), to produce biodegradable delivery “nanospheres”²⁸, which could be injected into the area where the connections are needed, under guidance with appropriate medical imaging (MRI or Ultrasound)²⁹. These would contain the NSCs and, when they deteriorate, would release them directly at the area of the required connection.

Alternatively, a chemical could be used which will encourage synapses to form. For this purpose, one possibility is a protein called **Reelin**, for which “preliminary data suggests...specifically regulates dendritic development”³⁰ this would mean that the chances of synapses corresponding would increase, and so make it more likely for the cells to be able to transmit impulses across the synaptic cleft.

Once the white and grey matters are synthesised to produce the body of the cord, any nerve roots required would need to be produced. These could be cultured and then implanted into the growing white and grey matter; the connections could be

stimulated with Reelin, as suggested above. To produce the meninges, cells from the meninges in the undamaged areas of the spinal cord could be cultured and grafted on to the new structure whilst still *in vitro*. Cerebrospinal Fluid (CSF) would not be injected until the cord was transplanted into the patient's body.

The tissue must also be vascularised by either grafting on tiny vessels, so that when implanted the vessels can be joined with the major vessels in the body, as seen in modern micro-vascular surgery, or by allowing the tissue to revascularize alone³¹. This vascularisation could be stimulated using FGFs, which encourage angiogenesis, as described above. This if injected to the cord after transplantation (epidurally), would assist vascular growth in the area, as well as also helping to differentiate the NSCs into neural and glial cells³².

In order to implant this newly cultured section of spinal cord a very major operation would be needed. The vertebrae around the area damaged by the trauma would have to be reposition allowing removal of the damaged section of cord. To ensure that sufficient synaptic connections were made in the new cord, the nanospheres containing the stem cells and a quantity of Reelin would be injected by an epidural needle, into the space at the sites where the new cord would join with the old. This would increase chances of the production of the synaptic linkages³³. After joining spinal nerves, in the same way, to their corresponding nerves injecting the above mentioned mix, large amounts of muscular function could be restored. A recent study showed that "NSCs transplantation resulted in extensive growth of corticospinal axons and locomotor recovery in adult rats after complete spinal cord transaction"³⁴. This would suggest that the technology could be used to the same effect in humans.

Method 2

The second solution is to inject the stem cells prior to their growth onto the damaged area of the spinal cord via a needle piercing the meninges and arriving in the damaged area. To ensure that the repair is completed by the stem cells, a mixture of chemicals would need to be designed to stimulate appropriate production of neurons and glia in the damaged area. A method of injecting this cocktail of chemicals and stem cells into the spinal cord, without causing further damage, is also required.

The stem cells would need a variety of chemicals to optimise their growth, as described previously. Ezh2 for example, would be needed to ensure that when differentiating, the NSCs become predominantly neurons and oligodendrocytes. This would allow the cord to produce new axons with myelin sheath covers to aid transmission of impulses. As well as stimulating nerve cell growth, chemicals which cause vascular growth in the new tissue (such as FGFs) are also needed for the differentiated cells to be supplied with oxygen and other substances vital for life such as substrates to allow protein synthesis within the cells.

The NSCs would be contained inside PLGA nanospheres, to ensure that they develop in the right location.

Our suggestion for the implantation of the stem cells is to use a similar system to an epidural catheter. The catheter would be placed using an epidural needle. This would have to pierce the meninges to reach the affected area of the spinal cord, so would be placed with the guidance of MRI or Ultrasound. The catheter could then be inserted through the needle to allow direct delivery of the stem cells and NGFs onto the area of damage. The catheter could be attached to an IV bag containing the components described above, to allow constant feed directly to the affected area.

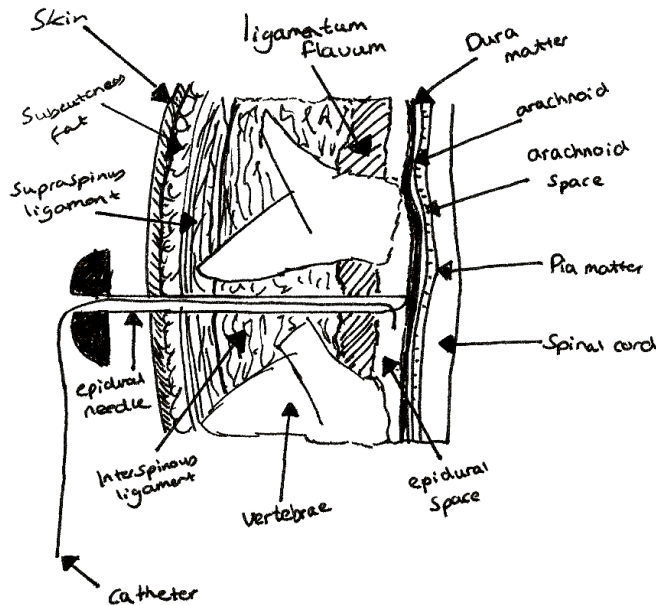


Figure 6: diagram of epidural needle with catheter placed in epidural space prior to final insertion into the damaged region of the spinal cord.

A Source of Stem Cells

This subject has hosted controversial debates since the very first research programs into stem cells began. The best sources of stem cells are aborted embryos, due to the pluripotent nature of embryonic stem cells which makes them most suitable for regenerative medicine, though major religions have condemned this as murder.

However, recently scientists in Edinburgh have been investigating the use of genes to cause normal epidermal cells to revert to an embryonic-like state³⁵. Though this was previously possible through the use of viruses, the side effects meant that these cells were not suitable for medicinal use. Though the research is still in its early stages, it could open up possibilities for stem cells and their uses, which are “ethically acceptable” to people that are against using embryos. It would also eliminate the need for antirejection drugs because the cells could be harvested from the skin of the patient themselves, and so contain identical DNA to the other cells in the body.

Conclusion

Stem cells have great potential for treating spinal cord injury, and the development of the CNS in embryogenesis can be used as a model to discover more about this fascinating and mysterious area of medicine.

In the relatively near future, biodegradable nanospheres containing neural stem cells and the “cocktail” of chemicals described earlier could be implanted to allow some recovery of neural function, as has been observed in rodents already.

Though the idea of growing and implanting an entire new section of spinal cord is likely to still be a long way off, discoveries about the stimulating or inhibiting effects of chemicals, and the properties of stem cells themselves, show that it may yet be possible.

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