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Review Article

Bridging the gap: Spinal cord fusion as a treatment of chronic spinal cord injury

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ABSTRACT

Despite decades of animal experimentation, human translation with cell grafts, conduits, and other strategies has failed to cure patients with chronic spinal cord injury (SCI). Recent data show that motor deficits due to spinal cord transection in animal models can be reversed by local application of fusogens, such as Polyethylene glycol (PEG). Results proved superior at short term over all other treatments deployed in animal studies, opening the way to human trials. In particular, removal of the injured spinal cord segment followed by PEG fusion of the two ends along with vertebral osteotomy to shorten the spine holds the promise for a cure in many cases.

Keywords: Electrical stimulation, GEMINI, polyethylene glycol, spinal cord fusion, spinal cord transection

To L. Walter Freeman, in memoriam

Those who cannot remember the past are condemned to repeat it.

George Santayana

TREATMENT OF SPINAL PARALYSIS: STATE-OF-THE-ART

Spinal cord injury (SCI) in man often leads to severe permanent disability. Ever since the work of Ramon and Cajal,[102] long-distance regeneration of injured axons across an injured segment of the cord has proven elusive. The limited regenerative capacity of the adult mammalian spinal cord has been attributed to the formation of cavities (cysts) and scarring that interrupt the ascending and descending pathways, low intrinsic regenerative state of injured neurons, and unfavorable microenvironment, such as an inhibitory extracellular matrix (ECM) that develops around the site of injury, inhibitory myelin-associated proteins (e.g., Nogo-A, MAG, and OMgp) and a lack of growth-promoting factors, such as neurotrophins.[32,120]

Several therapeutic strategies have been deployed over the past 40 years in experimental animals, with a focus on cell grafts, particularly grafts of various types of stem cells, into the injury site, to form a neuronal relay circuit across the gap.[6,31,34] A neuronal relay calls for synapse formation between the host extending axons from the rostral area to the injury/graft site and the donor neurons in the injury/graft site of spinal cord, appropriate release of neurotransmitters of the donor neurons, extension of axons from the donor neurons to areas caudal to the injury/graft site, and finally synapse formation between the donor extending axons and the host neurons in areas caudal to the injury/graft site. Both remyelination of axons across the lesion and generation of new neurons are necessary to achieve these goals.[6,31,34]

Spurred by promising animal studies, clinical trials of a wide variety of different cell lines implanted at or around the lesional level (Schwann cells – SC, olfactory ensheathing glia – OEG - residing either in the lamina propria or along the nerve fiber layer of the olfactory bulb, mesenchymal/stromal stem cells – MSC, some of which may acquire neuronal properties, multipotent progenitor cells – MPC, neural stem/progenitor cells – NSC, embryonic stem cells – ECS, and umbilical cord blood cells) have been (and are being) conducted over the past 20 years.[6,31,34] No biological cure defined as independent, permanent, and unaided deambulation has been achieved to date. Some open-label, uncontrolled reports claimed positive effects, even years after the injury, with some patients walking again for short distances with braces and support (although far from *restitutio ad integrum*).[27,59,103] However, negative studies and complications are equally on record.[1,6,28,31,34,113] Scaffolds in combination with cell grafts have been implanted, but early results do not seem especially promising.[134]

In sum, while some benefit may accrue from cell grafts and other techniques, they alone cannot cure paralysis.[132] As emphasized recently, *"it would be difficult to find any other branch of science with over a century of such sterile endeavour. In effect, there has been repetition of the same idea, albeit with different techniques, that is, looking at the lesion site. Are we sentenced to repeating the same experiments in the hope of expecting a different result?"*[55]

In this paper, we will review the evidence supporting an idea posited half a century ago by the US neurosurgeon L. Walter Freeman, namely that a permanent, biological cure is possible in several cases, by cutting out the most damaged portion of the spinal cord and connecting the two free ends, after spinal shortening [Box 1].[42] One should notice that removing the epicenter of a damaged cord and then connecting the two fresh ends is akin to reconnecting a transected spinal cord tout court. The process would be spearheaded by the use of so-called fusogens (GEMINI protocol).[21] Another group recently upheld this same concept.[94]

SPINAL CORD TRANSECTION: NATURAL HISTORY

In man, no recovery follows spinal cord transection (SCT) at whatever level as seen, for example, after stab wounds.[26,74,77,105,112,117] When the transection is partial, recovery is possible: 66% of 450 patients with stab wounds could eventually walk without or with only minimal help in one series and over half of 217 patients returned to their former occupation, usually within 6 months of the injury, in another.[4,84] Brown-Sequard types of lesion (i.e., hemisections) also recover: for instance, two patients with cervical hemisection recovered walking at 10 and 2 years[35] and another recovered almost completely at 3 years.[33] If the section is >50% (of a hemisection), results are similar to a complete section: in a representative patient, whose spinal cord was almost completely divided at C7/T1, only sensory disturbance was slightly improved at 4 months after the injury.[126]

A similar assessment applies to experimental animals. Handa *et al.*[49] performed a T9–10 SCT on 9 adult female dogs. Follow-up lasted 6–39 months. Within several weeks, muscle tone of the hindlimbs was gradually increased accompanied by the development of flexion reflex with after-discharge in addition to monosynaptic reflexes. Alternating stepping movements also began to develop. Afterward, extensor thrust and crossed extension reflex were observed. Standing behavior of the hindlimbs was found after sufficient development of the extensor thrust and correct placement of the pads of the toes. Steady development of stepping and standing caused forward locomotion using fore – and hindlimbs; 7 out of 9 could walk on open ground. This ability of locomotion by the hindlimbs of the spinal dogs reached a plateau 6 months after the surgery. Walking behavior of the hindlimbs was not inhibited by additional SCT in the two dogs where it was done, pointing to spinal automatisms and development of responses induced by afferent inflow from outside the cord as the reason for such functional recovery. This was corroborated by the electrophysiological absence of conduction across the transection. Veterinary experience shows that a section >50% at C5–6 in dogs is lethal,[12] unlike hemisections.[78]

Rodents follow a similar pattern. In untreated mice with dorsal SCT, 33% displayed weak nonbilaterally alternating movements (NBA) at 1 week. At 2 weeks, increased NBA were observed and the first BA movements in 10% of the animals. A progressive increase of movement frequency and amplitude was found after 2–3 weeks. By the end of the month, 86% displayed mixed NBA and BA. However, none of them recovered the ability to stand or bear their own weight with the hindlimbs.[47] On the Basso-Bresnahan-Beattie (BBB) scale,[10] a successor of the Tarlov's open field test, recovery from dorsal SCT in rats is no better than 3 out of 21 points at 6 weeks.[11] Rarely, scores of 5 have been reported, but these do not signal useful recovery, even if higher than controls [Table 1]. Conversely, most hemisection and contusion injury SCI models exhibit high rates of spontaneous recovery of locomotion[111] and are thus of dubious translational significance. In monkeys submitted to C7 hemisection, locomotor recovery is also fairly extensive.[110] In sum, in mammals, SCT leads to unrecoverable paralysis.

Box 1: Walter freeman and the cure of paralysis.

In the early years of the 20th century, Stewart and Harte[124] reported on CN, aged 26 years, who had her spinal cord severed by a *0.32 caliber* gunshot. The distance between the segments of the cord was 0.75 inch, as verified by all five attending physicians: "*The ends of the cord were then approximated with 3 chromicized catgut sutures passed by means of a small staphylorraphy needle, one suture being passed anteroposteriorly through the entire thickness of the cord and the other two being passed transversely. This part of the operation was attended with unusual difficulties because of…the wide interval between the fragments, the catgut frequently tearing out before the ends were finally brought together*." 16 months later, "*the patient slides out of bed into her chair by her own efforts and is able to stand with either hand on the back of a chair, thus supporting much of the weight of the body*." Although their specific conclusions were later mooted, they reviewed several cases of patients with sharp wounds to the cord that spontaneously recovered from initial paraplegia. Their conclusion was that "*the operation of myelorrhaphy will be specially indicated in cases in which the cord has been cut by a sharp instrument or severed by a projectile*."

It was in this spirit that the US neurosurgeon L. Walter (Bill) Freeman undertook to cure spinal paralysis on return from his World War II military service. In his canine (mostly female dogs) experiments, SCT was confirmed by lifting up both ends of the cord so that its cross-section could be seen in both its superior and inferior ends. Thereafter, he suspended paraplegic dogs in slings which protected the dogs from damage to paralyzed, insensitive extremities, and let dogs move freely on smooth surfaces covered with clean sawdust. Bladders were emptied by gentle pressure 3 times each 24 h for about 2 weeks, until bladder and bowel functioned automatically. All animals were fed a high protein diet, which created the right milieu for recovery. Yet, the key to success turned out to be devoted care by laboratory personnel, a fact leveraged by a Japanese group in 2015 as they proposed deep brain stimulation of the nucleus accumbens to enhance "motivation" (will-power) in spinally injured patients.[115] In this way, Freeman showed the return of function in hundreds of rats, cats, and dogs, with less success in monkeys, which are much harder to keep alive, although here too electrophysiologic conduction was demonstrated across areas of SCT. On microscopy, many axons in the proximal spinal cord above the transection grew toward the isolated distal segment below. He wrote:[42]

"Occasionally, a paraplegic rat would walk several months after (sharp) cord transection.in the area of transection, numerous growing axons from such a walking rat are shown…when we were able to maintain adult dogs in good health for long enough periods of time, they too showed functional return… liberal growth of axons from viable neurons in the spinal cord which has penetrated the area of transection and has established function. Furthermore, they show conduction of electrical impulses."

To improve results, he first employed X-ray therapy at the site of SCT to help regrowing axons extend past the scar tissue barrier into the distal cord. Thereafter, as suggested by Professor Donald Bowman, he instilled trypsin intrathecally to the site of transection through externalized thin plastic tubes: Many animals treated with trypsin prepared by Bowman's method showed significant return of function after SCT, including jumping on previously paralyzed hind legs (IM trypsin injected intramuscularly for several days after spinal cord section provided the same benefit). Axons regrew from the proximal spinal cord, past the area of diminished scarring into the distal segment of the isolated spinal cord, while in controls growing axons were blocked by dense scarring. Most importantly, Freeman noticed that return of function after SCT could only be due to growing axons synapsing with motor neurons in the distal segment of the spinal cord.

He thus concluded [**Figures 1b and c**]:[42]

"Realizing that the average clinical injury to the spinal cord is not a sharp surgical transection such as that which we used in the early experimental procedures, but instead a broad, long lesion, we set out to devise surgical procedures to duplicate these circumstances. To bring fresh ends of the divided spinal cord together, we resected enough vertebral body and thus shortened the spine. The damaged area could be removed, and by suturing the dura mater, we could approximate the fresh ends of the spinal cord. Walking animals resulted from this procedure, and axons grew through the area where the cord resection and anastomosis had been conducted."

Freeman also conceived and carried out the first implantation (embedding) of intercostals nerves into the rostral or caudal ends of the cord above or below SCT to act as bridges for regenerating fibers: Again, function returned in many cases. Clinical series confirmed these results.[141]

SPINAL CORD TRANSECTION: EXPERIMENTAL TREATMENT IN ANIMALS

It is clear from the above section that SCT lends itself as the ideal model to study neuroregenerative strategies. However, marked differences exist between human and rodent spinal cords both in anatomy and secondary injury processes,[32,43,91,137] while strong similarities exist between humans and dogs.[86,131] Unfortunately, canine studies of SCT, despite their greater translational relevance, are sparse. One has, thus, to bank on rodent studies in selecting promising translational avenues. The outcome in rodents is often plotted on the BBB scale (above), which allows comparisons among treatments at different time points. Ideally, a promising rodent study will show strong recovery within a very short time-frame. Unfortunately, the vast majority of published studies report useful recovery – when positive – after up to 2–9 months [Table 1]. Since 1 rat month is comparable to 3 human years,[116] translation to the clinic would imply many years before any effect is seen in man. It is thus imperative that we consider only the extent of recovery at no >1 month and then evaluate the effect in larger animals.

As can be seen from Table 1, acutely deployed (i.e., immediately after SCT) polyethylene glycol (PEG) fusion is superior to any other acute strategy published to date, including various cell grafts, conduits, and gene therapy. At 4 weeks no other technique approaches the extent of recovery seen with PEG fusion. This result has been corroborated by independent replication in separate laboratories in Japan, Korea, and China. Remarkably, PEG is

Table 1: Summary of behavioral outcomes of controlled studies utilizing the Basso-Bresnahan-Beatty scale after complete spinal cord transection in rodents and associated therapeutic interventions.

Authors	References	Level of transection	Intervention (S)	Outcome (BBB) at end of follow UP	Outcome at 4 weeks
Rapalino *et al.*	Nat Med 1998;4:814	T8-9	Macrophages pre-exposed *ex vivo* to peripheral nerves+aFGF	Tx: 7.5 (max 8) Ctrl: 1 19 weeks	Tx: 2 Ctrl: 1
Liu *et al.*	J Mol Neurosci 2013;51:629	T10-11	Electroacupuncture	Tx: 8 Ctrl: 6 5 weeks	NA
Wang *et al.*	Med Sci Monit 2017;23:4241	T10-11	Electroacupuncture		Tx: 5 Ctrl: NA
Li *et al.*	Neural Regenerat Res 2015;10:1317	T10	Panax NotoGinseng single IV injection 30' after section (to increase NGF/BDNF)		Tx: 6.22±0.77 Ctrl: 4.36±0.77 30 days
Zhang *et al.*	Spinal Cord 2007;45:496	T8	40 days of weight supported treadmill training	Tx: 7 (never>10 at all-time points) Ctrl: 2 45 days	Tx: 6 Ctrl: 2
Li *et al.*	Front. Cell Neurosci 2017;11:381.	T9 2 mm aspiration gap	recVEGF IP	Tx: 5 (max 6) Ctrl: 1 6 weeks	Tx: <5 Ctrl: 1
Bai *et al.*	Eur J Physiol 2010;460:657	T10	-ChABC in gelfoam into lesion site (A) -Clenbuterol (B)	Best result: A+B 4 (all: never>5) (Ctrl: <2) 12 weeks	Tx: 1.5 (<2) Ctrl: ≈ 0
Erceg *et al.*	Stem Cells 2010;28:1541	T8	Human embryonic stem cells differentiated into oligodendrocyte (A) or motoneuron (B) progenitors (MP/OP)	Best result: MP+OP 10 (Ctrl: <2) 17 weeks	Tx and Ctrl: ≈ 1
Kang *et al.*	Biomaterials 2012;33:4828	T8-9 2 mm aspiration gap	PLGA scaffold+human MSC (SCR1/2/3)	SCR 1: 4.5 SCR 2: 5 SCR 3: 6 (high dose) Ctrl: 2 8 weeks	NA (At 2 weeks: All groups 3)
Cheng *et al.*	PLoS One 2015;10:e0138705	T8	Low versus High Dose Chondroitinase ABC (intraparenchymal inject)	Best Tx (high dose): 3.4±0.9 Ctrl: 0.75±0.2 10 weeks	Tx : 2 Ctrl : ≈ 0
Ziemlinska *et al.*	PLoS One 2014;9:e88833	T9-10 T11-12	Adeno-associated Virus (AAV) vector expressing BDNF, single injection bilat. within 30' below section (L1)	Modified 0-22 BBB Assessment with or without TS Tx: 2 dead/13.7±5.14 (no TS)/14.8±2.68 (TS) Ctrl: No TS: 2.3±0.58 TS: 13 37–47 days	NA Tx: 10.7±6.13 (TS: 15.4±4.8) Ctrl: No TS: 0 TS: 4.5±0.71 2 weeks
Miura *et al.*	Exp Neurol 2000;166:115	T10 1 mm aspiration gap	AAV vector injected into both stumps near section expressing MEK-1 (activator of neurotrophin cascade)	Tx: 5.8 (4 out of 11: <3) Ctrl: <3 6 weeks	Tx: 4 Ctrl: ≈ 1
Liu *et al.*	Mol Neurobiol 2014;50:1035	T10	Lentivirus to upregulate Erp29 injected into motor cortex	Tx: 3.5 Ctrl: <2.5 8 weeks	Tx: 4 Ctrl: <2.5
Cen *et al.*	Spine 2013;38:1632	T10 2 mm aspiration gap	Lentivirus+Lingo-1 blocker injected into gap	Tx: 9 (up to 11) Ctrl: <3 8 weeks	Tx: 5 (max: 7) Ctrl : 2
Rooney *et al.*	Tissue Eng (Part A) 2011;17:1287	T8-9 2 mm gap	Oligo PEG-fumarate hydrogel scaffold embedding 1-dbcAMP encapsulated in PLGA microspheres 2- MSC or SC		Best result: MSC+camp microspheres Tx: 6.5 Ctrl: ≈3

(Contd...)

Table 1: *(Continued)*

Authors	References	Level of transection	Intervention (S)	Outcome (BBB) at end of follow UP	Outcome at 4 weeks
Nomura *et al.*	Neurosurg 2006;58:183	T9 2.5 aspiration gap	Coil-reinforced PHEMA or PHEMA-MMA channel (methacrylate) + cocktail of autologous peripheral nerve grafts, fibrin matrix, and aFGF	Best Tx (combo): 4 (max 5) Ctrl: 2 16 weeks	All Tx=Ctrl 1.5
Chen *et al.*	Sci Rep 2015;5:9017	T9 2 mm aspiration gap	Microporous hydrogel soaked in bFGF embedded in an acellular vascular matrix inserted 5 days after a section	Tx: 13 (<15 at all timepoints) Ctrl: 7–8 8 weeks	Tx: 9 Ctrl: ≈1
Liang *et al.*	Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2009;23:1376	T9	BMSCs seeded on the denuded human amniotic membrane, BMSCs-DHAM	Best Tx: 12.50±1.26 3 months	NA
Pal *et al.*	Int J Nanomed 2013;8:2259	T11	Iron oxide nanoparticle+gel into gap+Electromagnetic field (50 Hz, ≈18 μT, 2 h/die, 5 weeks)	Best result (full combination) Tx: 8 (MF: 5.5; NP: 4) Ctrl: 3 5 weeks	NP+MF: 6 NP: 4 MF: 5.5 Ctrl: 3
Luo *et al.*	Acta NCH 2009;151:1483	T9-10	BMSCs injected into gap±subcutaneous G-CSF for 5 days	Best result (cells+G-CSF) 10 (max. 12 at all time points) Ctrl: ≈4 8 weeks	Tx: 5 (max 6) Ctrl: ≈3
Yang *et al.*	Plos One 2008;3:e3336	T7-9 1–2 mm gap	Human umbilical MSC (Wharton's jelly) (MSC)±treatment in NCM for 3–6 days with fibrin glue injected into gap plus into both stumps	Best result (MSC only without NCM) 6.96±0.26 (Ctrl: 0.88±0.2) 8 weeks	Tx (MSC): 4.81±0.29 Ctrl: 0.88±0.2
Zeng *et al.*	Biomaterials 2015;53:184	T9-10 2 mm aspiration gap	BMSC (with neuronal differentiation) engineered to express NT-3 receptor and differentiated into neurons by co-culture with NT-3-producing SC injected into gap acutely	Tx: 8.85±2.03 Ctrl: 3 8 weeks	Tx: 6 (max 8) Ctrl: 3
Buzoianu-Anguiano *et al.*	Neural Plasticity Volume 2015, Article ID 389520	T9	PPN BMSCs	Best Tx (combination): 4 Ctrl: <1 2 months	Tx: ≈3 Ctrl: <1
Qiu *et al.*	Stem Cell Res Ther 2015;6:105	T9-10 2 mm aspiration gap	MSC neuronalized by overexpression of NT-3 receptor or NT-3 gene+GS scaffold	Tx: 5 (<6) Ctrl: 2 (<3) 8 weeks	Tx: 3 (<4) Ctrl: <2
Sharp *et al.*	Exp Neurol 2014;257:186	T3 1–1.5 mm gap	Fetal Neural stem Cells in fibrin matrix+growth factors cocktail±scar removal	Tx: 5 Ctrl: 1 7 weeks	Tx: max 5 Ctrl: max 2
Li *et al.*	Cell Mol Neurobiol 2011;31:407	T9 2 mm aspiration gap	Neural Stem Cells (NSC) suspension+injection rostrally/caudally acutely OR after 7 days		Best Tx (subacute, rostral): ≈6 (acute rostral: in one rat, 7) Ctrl: 4
Zhang *et al.*	J Neurotrauma 2007;24:1863	T10	Un/Predifferentiated NSC OR SC suspended in scaffold	Best Tx (predifferentiated NSC): 6 (max 7.5) Ctrl: <1 8 weeks	Berst Tx (und. NSC): 2 Ctrl: <1

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Table 1: *(Continued)*

Authors	References	Level of transection	Intervention (S)	Outcome (BBB) at end of follow UP	Outcome at 4 weeks
Zhang *et al.*	Neurochem Res 2009;34:2030	T9-T10 1–2 mm gap	Human umbilical (Wharton's jelly) MSC-derived neurospheres±BDNF	Best Tx (combination): ≈7 (<8) Ctrl: <2 10 weeks	Best Tx: <3 Ctrl: <2
Tian *et al.*	Biomater Sci 2017;5:2480	T8-9 2 mm aspiration gap	Acellular PNG+placenta MSC	Best Tx (combination): 13 Ctrl: <3 8 weeks	Best Tx: 8 Ctrl: <3
Lu *et al.*	J Neurosci 2012;32:8208	T3	Syngeneic bone marrow stromal cell (MSC) graft in the lesion site, gradients of BDNF within and beyond the lesion site, and cAMP injections into the brainstem BDNF engineered in MSC Injections of viral vectors expressing BDNF, 1.5 and 2.5 mm caudal to the lesion site-cAMP administered directly into pons	Tx (combination): Max 8 Ctrl: 1 3 months	NA
Lu *et al.*.	Brain Res 2001;898:344	T10 1–2 aspiration gap	OEG suspension in gelfoam into stumps+OEG pieces into gap	Tx: 5–6 Ctrl: <2 8–10 weeks	Tx: 2 Ctrl: 1
Lopez-Vales *et al.*	Neurobiol Dis 2006;21:57 Neurobiol Dis 2006;24:443	T8	OEG (olf. bulb) cells suspension, multiple injections into both stumps 30' after section (acute) or delayed±FK506	Best Tx (acute): 4.2±0.7 (if motor evoked potentials present: 5.3±0.5) Ctrl: Max. 2 9 months Best Tx (+FK506): 5.1±0.76 Ctrl: <2	Tx=Ctrl (<1) 30 days As above
Lee *et al.*	J Neurotrauma 2002;19:1203	T8 5 mm aspiration gap	-aFGF+fibrin - PNG	Best Tx (combination): 6.5–7 Ctrl: ≈0 6 months	Tx: 1 Ctrl: ≈0
Lee *et al.*	J Appl Physiol 2007;103:1808	T8 5 mm aspiration gap	PNG	Tx: max. 7 Ctrl: max. 3 6 months	NA
Kuo *et al.*	J Neurosci 2011;3:4137	T8 5 mm aspiration gap	autologous peripheral intercostal nerve segments+aFGF in a fibrin glue carrier	Tx: 4 (max.) Ctrl: <1 8 weeks	Tx: 2.5 Ctrl:<1
Tsai *et al.*	J Neuropath Exp Neurol 2005;64:230	T8 4 mm aspiration gap	-autologous intercostal nerves inserted 0.5 mm into stumps+fibrin glue+FGF -anastomosis+nerves fibrin-glued around cord	Tx: Grafts: 4.13–8.13 (max 9.5) Anastomosis: 4.13–9.38 (earlier and better recovery) Ctrl: 0-38–2.38 13 weeks	Tx (all): <3 Ctrl: NA
Cruz *et al.*	J Mater Sci 2012;23:2583	T9	Plasma Polypyrrole scaffold implants (PPY±PEG)	Best Tx (with PEG): 4.6–4.7 (4.5.5) Ctrl: 2.2	Tx: ≈3 Ctrl: ≈1.5

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Table 1: *(Continued)*

Authors	References	Level of transection	Intervention (S)	Outcome (BBB) at end of follow UP	Outcome at 4 weeks
Olson *et al.*	Tissue Eng (part A) 2009;15;1797	T8-9 2 mm gap	Neural stem Cells Scwann Cells PLGA scaffold		Tx (NSC): 1.92±0.43 SC 1.14±0.45 Ctrl: 0.96±0.04
Luzzi *et al.*	Surg Neurol Int 2018;9:19	T9	Heterologous bovine marrow MSC	Tx: ≈9 (2 rats: 14) Ctrl: 1 70 days	Tx : 7 Ctrl: 1
Xiong *et al.*	Front. Cell. Neurosci 2017;11:213	T10	Hematopoietic stem cells (HSCs) transplanted intraspinally into the rostral, scar, and caudal sites of the transected lesion at 14 days post-operation	Tx: 9 (max 10) Ctrl: 6 Weeks 24	Tx : 4 Ctrl: 2
Reynolds *et al.*	Spinal Cord 2000;46:58	T10 2 mm aspiration gap	Porous methacrylate derived tube		Tx: 7.1 Ctrl: 1.4
Blesch *et al.*	J Comp Neurol 2003;467:403	T7	GDNF-secreting fibroblasts	Ctrl>Tx (3.3 vs. 2.7) 3 months	Ctrl>Tx
Centenaro *et al.*	Brain Res 2011;1426:54	T8-9 2–3 mm gap	OEG Injected acutely, at 2 weeks, at 4 weeks	Tx≈/<Ctrl 80 days	Tx≈ctrl (3)
Steward *et al.*	Exp Neurol 2006;198:483	T10	OEG 30 days post-injury	Tx=Ctrl (0.5) 3 months post-graft	Tx=Ctrl (<1)
Lu *et al.*	Brain 2002;125:14	T10 3–4 mm gap	OEG	Tx: 4.3±0.8 Ctrl: 1±0.2 10 weeks	NA
Yan *et al.*	Zhonghua Wai Ke Za Zhi 2009;47:1817	Low Thoracic	GDNF modified olfactory ensheathing cells (OEGs) combination with injecting axonal growth inhibiting protein antibody (IN-1)	Ctrl: 7.70±0.24 (!!!) In-1: 7.89±0.15, OEG: 10.50±0.25, GDNG-OEG. 11.43±0.23 Combination: 12.81±0.40 8 weeks	NA
Fouad *et al.*	J Neurosci 2005;25:1169	T8 4 mm aspiration gap	-Channel containing Matrigel and SC in gap -OEG injected in stumps -Chondroitinase (cABC) infused through pump into stumps	Tx (grafts): 4±0.6 Tx (cABC): 6.6±0.7 Ctrl: 2.1±0.7 9 weeks	Tx (all)=Ctrl 1
Lukovic *et al.*	Sci Rep 2015;5:9640	T8-9	Human embryonic stem cells	Tx: ≈6 Ctrl: ≈1.5 Week 17	Tx: 1.5 Ctrl: 0.5
Ganz *et al.*	Front. Neurosci. 2017;11:589.	T10	PLLA/PLGA Scaffold+induced/naïve human oral mucosa stem cells	Best Tx: 11 (max: 19-20) Week 13 Ctrl: 2 Weeks 7	Best Tx: 9 (max 12) Ctrl: 1
Madigan *et al.*	Tissue Eng (Part A) 2014;20: 2985	T9	Oligo-PEG-fumarate Scaffold loaded with MSC or SC		Tx (SC): 5±0.97 Tx (MSC): 3.81±0.77≈Ctrl: 3.81±0.76
Chen *et al.*	J Tissue Eng Regen Med 2018;12:e398	T9	Positively-charged oligo[poly (ethylene glycol) fumarate] + SC or SCs genetically modified to secrete high concentrations of GDNF		Best Tx: 3.67±0.40 (GDNF-SC) 2.22±0.41 SC

(Contd...)

Table 1: *(Continued)*

Authors	References	Level of transection	Intervention (S)	Outcome (BBB) at end of follow UP	Outcome at 4 weeks
Liu *et al.*	PLoS One 2015;10 (3):e0117709	T10 2 mm aspiration gap	3D electrospun poly (lactide-co-glycolide)/ polyethylene glycol (PLGA-PEG) nanofiber scaffolds (2 mm long) seeded with induced neural stem cells (from fibroblasts)	Tx: PLGA/PEG: 17 (max. 19) PLGA: ≈15 (max. 16) Ctrl: 7 (max. 8) 10 weeks	Tx: PLGA/PEG: 11 (max. 13) PLGA: 9 (max. 10) Ctrl : <5
Oda *et al.*	J Vet Med Sci 2014;76:415	T10	BMSC injected in both stumps acutely OR PEG 4000 injected acutely rostral and caudad OR Combination (PEG injected into lesion site)		Tx BMSC=PEG 4000=Combination≈12 (max 13) Ctrl: 8
Ye *et al.*	Surgery 2016;160:20	T10	PEG 1500 PEG 4000 saline		Tx: PEG 1500: 14 PEG 4000: 10 Ctrl: 2
Ren *et al.*	CNS Neurosci Ther 2017;23:680	T10	PEG 600		Tx: 12 (in 2 rats: 19 and 20) Ctrl: 4.4
Kim *et al.*	Neural Regener Res 2018;14:1440	L1	Graphene nanoribbons+PEG 600	Tx: 8 (max 9) Ctrl: <4 5 weeks	Tx: 7 Ctrl: <4
Koffler *et al.*	Nat Med 2019;25:263	T3 1.8 mm removed	3D printed 2 mm PEG-GelMa scaffold, E14 NPC in fibrin, BDNF, VEGF, bFGF, calpain inhibitor	Combo Tx: 6.6 +/-0.5 Empty scaffold: 0.3+/-0.2 Scaffold + NPC: 1.6+/-0.8 20 weeks	Combo Tx: 4 0 1
Shu *et al.*	Neurosci Letters 2019;602:33	T9 2mm removed	Polylactic acid +/- Polypyrrole (conductive) scaffold	Combo Tx: ≈5.5 No Polypyrrole: 3 No Tx: 1.5 6 weeks	Combo Tx: 4 (max 5)
Hakim *et al.*	J Tissue Eng Regen Med 2019 (in press)	T8 2mm gap	PLGA microspheres, rapamycin, Schwann cells, Oligo-PEG/fumarate scaffolds, with/without OPF	Tx: max 6 6 weeks retransection	Best combo Tx: ≈5

NB: All studies refer to rats, except Oda *et al.* and Ye *et al.* (mice). OEG: Olfactory ensheathing glia. BBB scale: 0=paralysis of hind limbs, 21=normal gait. Scores from 1 to 7 (Level 1) mark the return of isolated movements of hip, knee, and ankle, scores from 8 to 13 (Level 2) the return of hindlimb coordination, and scores from 14 to 21 (Level 3) the recovery of predominant paw position, trunk stability, and tail position. MSC: Mesenchymal stem cells, SC: Schwann cells, NCM: Neuronal conditioned medium, PPN: Predegenerated peripheral nerve, BMSCs: Bone marrow stromal cells, PNG: Peripheral nerve grafts, GDNF: Glial cell line-derived neurotrophic factor, OEG: Olfactory lamina propria, TS: Tail stimulation, BDNF: Brain-derived neurotrophic factor, NGF: Nerve growth factors, PLGA: Polylactic-co-glycolic acid, 3D: Three-dimensional

inexpensive and easy to deploy, while most other technologies are labor-intensive and/or costly and/or highly specialized.

In the few canine studies, PEG fusion is again superior [Table 2]. For instance, Wu *et al.*[133] reported no motor function in the pelvic limbs at 15 days after the surgery (Olby score 0), with the gradual recovery of motor function of the pelvic limbs starting from the 1st month after stem cell grafting. On the contrary, in the PEG study,[80] motor recovery in treated animals began at 3 days (median cBBB score 2 vs. 0 in controls).

Even in monkeys, cell grafts are not especially promising, despite claims to the contrary in some papers. For instance, a grafting study of human fetal spinal cord-derived neural progenitor cells after C7 hemisection reported a >25% improvement in object manipulation scores in four of five monkeys (vs. 1 out of 4 controls that improved

so) and a 12% improvement in climbing score, beginning several months after grafting.[110] This is far from striking, and in line with clinical outcomes in man (see above); in addition, there was no lesioned sham control group, and monkeys with poor graft survival did not live as long as monkeys with surviving grafts. Instead, preliminary data suggest that PEG fusion is superior to this kind of grafts in a monkey model of SCT (manuscript in preparation).

It is worth mentioning that minimal retraction is seen after SCT and that in these cases PEG acts initially as a neuroprotectant (see below) and a bridge for regenerating axons across the gap. In the model suggested in this article, apposition is complete and PEG would also act as an axonal fusogen.[23] Thus, reported results of PEG fusion [Tables 1 and 2] represent an absolute minimum and these are expected to improve further both in terms of rate and extent of recovery once the severed ends of the cord are non-compressively approximated.

In conclusion, PEG fusion is an ideal candidate for a clinical trial.

UNDERSTANDING SCT

To understand the fusion process, one has to first understand the cellular processes in play in the setting of SCT.

Yoshida *et al.*[138] studied SCT in the rat. The sharpness of the transection turned out to be one of the most important factors for successful axonal regeneration. An extremely sharp transection produced edema-free lesions and later formed neither cysts nor scars, whereas a relatively blunt transection produced edema followed by scars and cysts around the lesions. Consequently, the spinal cord was transected using the edge of a razor which was as sharp as possible to minimize traumatic injury. However, the stump of the spinal cord developed edema, as in their model it took 10 or 20 min to bring together the two ends of the sectioned cord. This dovetails with a rodent study: the ends of the transected spinal axons remain stable for only about 10–20 min before undergoing fragmentation (the first step before classic Wallerian degeneration, or dieback) at both ends spanning 0.3 mm, only to stabilize and persist for 3–7 days; however, about 30% of proximal axons then start growing again within 6–24 h.[63]

Ramon and Cajal[102] already noticed "traumatic degeneration" in both stumps within 1 h of SCT in rabbits. Other studies showed that, immediately following SCT, axoplasm escapes from both the proximal and distal portions of some of the cut axons: the extent of the axoplasmic loss is generally greater in larger myelinated fibers. In contrast, small fibers, whether myelinated or unmyelinated, show little if any loss of axoplasm.1 h after SCT, the proximal and distal ends of the axons have retracted from the transection site, and both ends are separated by 1–2 mm or more from the transection site. The axoplasmic leakage stops within a few hours of the transection. Electron microscopic observations indicate that the tip of an axon is lined by axolemma within 1 h; in addition, layers of collapsed myelin form a septum in front of the axonal tip. At about 3 h after axonal transection, the axon becomes swollen and irregular in shape and massive accumulation of lysosomes and release of autolytic lysosomal hydrolases is observed within both the rostral and the caudal spinal cord stumps, peaking at 3–7 days and declining at 14 days: cavitation is the result.[38,62,95] Both the proximal and distal ends swell because axoplasmic transport is bidirectional. Degeneration spreads in both directions along the axon from the transection site, but only for a short distance in the proximal portion: in a clean cut, only one or two internodes may be involved within the proximal stump.[25] In the distal axon, however, Wallerian degeneration occurs.

In view of this data, it is obvious that whatever treatment must be brought to bear within minutes (<10).

FUSOGENS: THE ENGINE OF RECOVERY

Fusogens comprise a class of substances that have the capacity to reseal damaged cell membranes. Included in this class is PEG. PEG is a relatively inexpensive, stable, nontoxic, fully biocompatible, and water-soluble linear polymer that is synthesized by the living anionic ring-opening polymerization of ethylene oxide with molecular weights ranging from 0.4 to 100 kDa. It has a wide range of clinical and pharmaceutical applications, including, among others, an oral laxative, and several PEGylated drugs. PEG is FDA-approved for use as a preservative additive before organ transplantation to limit cold ischemia/reperfusion injury.[100] It does not accumulate in the body and crosses the blood–brain/spinal-barrier. It is considered immunologically inert, although anti-PEG antibodies have been detected in patients treated with and without PEGylated drugs, perhaps due to the widespread use of PEG in household products including toothpaste and shampoo.[44]

PEG has been shown to be strongly neuroprotectant thanks to its membrane sealing/fusing properties [Box 2].[70,83,108] PEG reduces both necrosis and apoptosis through two distinct yet synergistic pathways, i.e., repair of disrupted plasma membranes and protection of mitochondria through direct interaction. PEG may reduce the neuronal membrane tension and improves the membrane's fluidity so that sealing may occur, even in low-temperature conditions.[93,135] Interestingly, Nehrt *et al.*[93] noted that axons with small diameters preferentially benefited from PEG-mediated axolemmal resealing: many neurons of the truncoreticulopropriospinal (TRPS) meshwork (see below) are small-sized. Zhang *et al.*,[139] in a lamprey model, found that axon resealing is a critical determinant of neuron survival and the artificial acceleration of resealing with PEG reduced retrograde neuronal apoptosis by 69.5% at 2 weeks after SCI. They also reported that factors other than Ca++ diffusion into the injured tip contribute to retrograde death signaling and that the larger the neuron an axon belongs to, the slower the resealing.

Certainly, not all PEGs are created equal, and there is some evidence that molecular weight and other factors can influence the fusogenic

Table 2: Canine studies of spinal cord transection.

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
Han *et al.*	2014	24 adult female beagle dogs	T12 transection SHAM group (*n*=8) CTL group (complete spinal cord transection without any treatment (*n*=8) CSCB group (complete spinal cord transection with CSCB (*n*=8) 4–5 mm segment excised (7 mmgap) A 7 mm-long and 5 mm wide bundle of CSCB fibers binding with 5.6 nmol CBD-BDNF implanted in the gap and aligned with the spinal cord Collagen membrane placed over the two stumps to reduce peridural adhesion	Collagen scaffold-Collagen binding brain-derived neurotrophic factor (CBD-BDNF) complex (CSCB) Biocompatible LOCS (LOCS), bovine, each fiber 50–200 μm, made up of a number of finer micro-fibers A bundle of LOCS fibers (about 800–1000) incubated with 5.6 nmol of CBD-BDNF in 50 μl distilled water 1 h before implantation CBD-BDNF+ LOCS = CSCB	Olby score	4 weeks: 2.5 versus 1.5 8 weeks: <4 versus 2 12 weeks: 4.1 versus 2	CSCB 1-markedly inhibited the collagen deposition in the lesion center 2- the regenerated axons in CSCB implant originated from dorsal roots BUT NOT from cortico-spinal fibers; regenerated axons did not have obvious connection between the host spinal cord and the tissue in the graft Scaffold showed good biocompatibility, degradability and low immunogenicity	No imaging study	Spinal somatosensory evoked responses: CSCB group had markedly higher SSERs (72.7%±7.6%) than that (43.1%±3.3%) in CTL group
Wu *et al.*	2018	8–9kg /180–240-day-old adult female Beagle dogs adult female Beagle canines (6–8 months old, 8–9 kg, *n*=15)	1-MSC-derived neuron-like tissue and survived for 6.5 months (6.5 m- MT + SN group, *n*=6); 2- GS scaffold and survived for 6.5 months (GS group, *n*=3) 3- controls; survived for	T10 complete transection 4 mm spinal cord tissue plus corresponding paired spinal roots completely removed A 4 mm-long and 5 mm diameter of the MSC-derived neural network tissue	Olby scoring system (open field). Blinded evaluation of videos taken from 0.25, 0.5, 1, 2, 3, 4, 5, 6, and 6.5 months after the surgery Underwater treadmill submerging the body part 5 cm below the iliac crest (3.6 m/min;	Olby score 3–5: 6 DOGS (stage 2) 6–8 [8.3]: 5 DOGS (stage 3) all MT + SN 10 (stage 4): 1 DOG MT + SN No stage 5 voluntary tail movement: all	No residual host nerve fibers After co-culturing with NT-3-SCs in the 3D GS scaffold for 14 days, TrkC-MSCs exhibited phenotypic features resembling neurons Majority of MSC-derived cells at 14 days	MRI: low signal between the two ends of transected spinal cord at 3 days after surgery. DTI: Loss of integrity of nerve tracts in the injury/graft site. At 6.5 months after SCI: 4/6 animals with MSC-derived	Whole-cell patch clamp: a few cells gave off action potentials, but only TrkC-MSC cells seeded with NT-3-SC (not soluble NT-3): Post-synaptic currents recorded Canines receiving MSC-derived

(Contd...)

Table 2: *(Continued)*

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
			6.5 months (SCI group, *n*=3) 4-MSC-derived neural network and survived for 2.0 months (2.0 m-MT + SN group, *n*=3). BMSCs harvested from femurs and tibias and SCs harvested from sciatic nerves and brachial plexus of newborn male Beagle canines (1–3-day-old) Coculture of TrkC gene-modified MSCs and NT-3 gene-modified SCs in 3D GS scaffold 3-D GS scaffold (3D GS) with a 4 mm diameter and 5mm length Equal amounts of TrkC-MSCs and NT-3-SCs mixed and seeded to each scaffold (MT + SN) Daily supplement of 50 ng/ml human recombinant NT-3 protein (MT + NT-3) in a subgroup All canines	(MT + SN) or the GS scaffold GS grafted into the gap Dura loosely sutured at two knots in order to release any pressure that may arise in the post-injury edema phase Cyclosporin A (20 mg/kg) once daily till the end of the experiment.	5 min for recording circle) Blinded evaluation of videos at 0.5, 1, 2, 3, 4, 5, 6 and 6.5 months after the surgery MRI + DTI at 3 days and 6.5 months after surgery, MEPs at 2.0 m or 6.5 m after the spinal cord surgery IEM	-joint movements: 3 joint: 8 dogs (6 had MT/SN) -weight bearing: 1 >90% of the time; 5 <10%->50% of the time: all MT/SN None in 6 Underwater treadmill: constant or frequent alternate stepping in 6 dogs (all MT/SN); canines in the 6.5 m-MT + SN group regained about 20% time of coordinated front-pelvic limb locomotion (vs. GS group: <5% time and SCI group <2% time) In sum: MSC-derived neuronal graft: 7.60 ± 1.50, GS group: 4.20 ± 0.50 Controls: 3.70 ± 1.20 (6.5 months after surgery) Weight-bearing only in MSC-derived neuronal graft	after induction were immature neurons Both stem/progenitor and mature population were <40% 33% of cells presented coexistence of both NF-L and b-tubulin III, suggesting they were at the maturation process. Co-existence of SYP and PSD95 (27%) suggests that these cells had the potential to receive and deliver signals through synaptic transmission TEM: Synapse-like structures between MSC-derived neuron-like cells, including synaptic vesicles, synaptic cleft, distinct post-synaptic membrane thickening with enhanced electron density At 14 days after culture, a substantial number of cells expressed Nav1.7 (41.84% ± 7.86%) and KCND1 (37.34% ±10.05%). In prolonged *in vitro* culture up to 18 days, Nav1.7 and KCND1 positive cells rose to 86.08% ± 5.45% and 79.76%	neural network tissue showed a narrower gap between the two ends of transected spinal cord + nerve tract regeneration (vs. GS animals) 2/6 = GS controls	neural network had a shortened latency of MEP (33.70 ± 9.50 ms), as compared with that in the canines of the GS group (53.70 ± 4.10 ms). The improvement of latency of MEP began as early as 2.0 months after the graft of MSC-derived neural network tissue (35.40 ± 6.60 ms). There was no statistical difference in the amplitude and area of MEPs among the 2.0 m-MT + SN, 6.5 m-MT + SN and GS groups.

(Contd...)

Table 2: *(Continued)*

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
			sacrificed after 2 or 6.5 months (7.5 months for BDA injection ones) after the initial surgery				7.96%, respectively. MSC-derived neuron-like cells expressed GAD67 (GABA), glutamate and ChAT (Ach) Grafted cells survived up to 6.5 months, with most of them maintaining the expression of TrkC. Mature neuron population of donor cells in the rostral (4.72%± 0.48% vs. 0.32 ± 0.10%), central (12.93%±1.57% vs. 0.14%±0.07%) and caudal (8.39%±0.50% vs. 0.18%±0.08%) areas of the injury/graft site of spinal cord at 6.5 months after transplantation significantly higher than that in the corresponding areas at 2.0 months after transplantation. MSC-derived neuron-like cells bearing Nav1.7 channels, presynaptic marker SYP and postsynaptic marker (PSD95), some GABA and glutamate profiles		

(Contd...)

Table 2: *(Continued)*

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
							NB: Reactive astrocytes did not impede the growth of NF positive nerve fibers! EM showed a massive accumulation of collagen fibers in the injury/graft site in the 6.5 m-MT + SN group, but this did not seem to inhibit growth of cell processes and cell to cell contact 35 days after anterograde labeling of the motor neurons, descending M1 axons synaptically contacted MSC-derived neuron-like cells in the injury/graft site and may participate in the relay of motor cortex signals		
Liu *et al.*	2018	Female beagles (9 Kg) *n*=12	PEG 600	T10 7 dogs treated with PEG applied at the interface 5 dogs treated with saline	C-BBB (Scoring sessions occurred at 3, 10, 17, 24, 31, 38, 45, 52, and 59 days postoperatively) SSEP (before, during and 2 months post-operative) MRI – DTI (at 2 and 4 weeks post-operative)	PEG: Median: 8 (2 dogs scored 15 and 18) Controls: Median: 3 (max: 4) 2 months	Not done	Fiber regrowth at both timepoints (4 weeks > 2 weeks).	Almost normal wave configuration at 2 months
Liu *et al.*	2018, 2019	Female beagles (9 Kg) *n*=12	PEG 600	T10 7 dogs treated with PEG applied at the interface	C-BBB (Scoring sessions occurred at 3, 10, 17, 24,	PEG (2 months): Median: 8 (2 dogs: 15, 18)	At 6 months (n = 7): On HE stained sections (sagittal and	DTI: tissue reestablishment of anatomical continuity in PEG treated	At 2 months near normal waves Not done at 6 months

(Contd...)

Table 2: *(Continued)*

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
				5 dogs treated with saline 2 controls and 1 treated dog died between 2 and 6 months 2 dogs spared for long-term assessment (>1 year)	31, 38, 45, 52, and 59 days postoperatively) SSEP (before, during and 2 months post-operative) MRI – DTI (at 6 months post-operative)	Controls: 3 (max. 4) PEG (6 months): Median: 11 (2 dogs: 18 and 18) Controls: Median: 4 (max 5) Neuropathic Pain Assessment: no signs of undue subjective discomfort or frank pain behavior, indicative of the onset of central pain, were observed at 6 months and 1 year.	coronal), treated and untreated cords differed dramatically: Vacuolization due to tissue injury (cysts) was minimal in treated cords, with a highly significant difference with controls, a sign of the neuroprotective effects of PEG. On C-2R-B, myelin staining was abundant in PEG treated animals versus very little in controls. In controls, both above and at injury site, axons clearly showed massive signs of Wallerian degeneration, including where corticospinal fibers course; on the contrary, in treated animals, fibers were spared to a large extent and shown crossing the fusional interface through the scar. This was confirmed by immunolabeling neurofilament protein (NF200) that evinced remarkable axonal sprouting across the transection	animals as opposed to controls -near-normal dogs with almost normal appearing cords versus no *restitutio ad integrum* in untreated animals. At 2 weeks, 4 weeks and 6 months.	

(Contd...)

Table 2: *(Continued)*

Authors	Date	Animal	Study design	Treatment	Assessment	Outcome: Behavioral	Outcome: Histology	Outcome: Imaging	Outcome: Electro-physiology
							site. Some of these fibers also stained for 5-HT. No significant difference was seen in the amount of scarring in both groups of animals.		

SCs: Schwann cells, LOCS: Linear ordered collagen scaffold fibers, GS: Gelatin sponge, IEM: Immunoelectron microscopy, HE: Hematoxylin-Eosin, MRI: Magnetic resonance imaging

Box: Rating scales in dogs.

Olby score: Stage 1: 0–2 (0: No pelvic limb movement and no deep pain sensation; 1: No pelvic limb movement with deep pain sensation, and 2: No pelvic limb movement but voluntary tail movement), Stage 2: 3–5 (3: Minimal non-weight-bearing protraction of pelvic limb movement of one joint); 4: Non-weight-bearing protraction of pelvic limb with more than one joint involved <50% of the time; 5: Non-weight-bearing protraction of pelvic limb with >1 joint involved >50% of the time); Stage 3: 6–8 (6: Weight-bearing protraction of pelvic limb <10% of the time.; 7: Weight-bearing protraction of pelvic limb 10–50% of the time; 8: Weight-bearing protraction of pelvic limb>50% of the time), Stage 4: 9–11 (9: Weight-bearing protraction 100% of time with reduced strength of pelvic limb. Mistake >90% of the time, 10: Weight-bearing protraction of pelvic limb 100% of time with reduced strength. Mistake 50–90% of the time, 11: Weight-bearing protraction of pelvic limb 100% of time with reduced strength. Mistake <50% of the time), Stage 5: 12–14 (Ataxic pelvic limb gait with normal strength, but mistakes made >50% of time, 13: Ataxic pelvic limb gait with normal strength, but mistakes made <50% of time, 14: Normal pelvic limb gait).

Canine locomotor rating scale (cBBB) Score: 0=No observable HL movement 1=Slight movement of 1 or 2 joints 2=Extensive movement of 1 joint, or extensive movement of 1 joint and slight movement of 1 other joint 3=Extensive movement of 2 joints 4=Slight movement of all 3 joints of the HL 5=Slight movement of 2 joints and extensive movement of the third 6=Extensive movement of 2 joints and slight movement of the third 7=Extensive movement of all 3 joints in the HL 8=Plantar placement of the paw with no weight support 9=Plantar placement of the paw with weight support only when stationary, or occasional, frequent or consistent weight-supported dorsal stepping and no plantar stepping 10=Occasional weight-supported plantar steps; no FL-HL coordination 11=Frequent to consistent weight-supported plantar steps and no FL-HL coordination 12=Frequent to consistent weight-supported plantar steps and occasional FL-HL coordination 13=Frequent to consistent weight-supported plantar steps and frequent FL-HL coordination 14=Consistent weight-supported plantar steps, consistent FL-HL coordination, and predominant paw position is externally rotated when it makes initial contact as well as just before it is lifted off; or frequent plantar stepping, consistent FL-HL coordination, and occasional dorsal stepping 15=Consistent plantar stepping and consistent FL-HL coordination and no toe clearance or occasional toe clearance; predominant paw position is parallel to the body or internally rotated at initial contact 16=Consistent plantar stepping and consistent FL-HL coordination and toe clearance occurs frequently; predominant paw position is parallel or internally rotated at initial contact and externally rotated at liftoff 17=Consistent plantar stepping and consistent FL-HL coordination and toe clearance occurs frequently; predominant paw position is parallel or internal at initial contact and at liftoff 18=Consistent plantar stepping and consistent FL-HL coordination and toe clearance occurs consistently; predominant paw position is parallel or internal at initial contact and at liftoff. Trunk instability is present 19=Consistent plantar stepping and consistent FL-HL coordination and toe clearance occurs consistently during forward limb advancement; predominant paw position is parallel or internal at initial contact and liftoff. Trunk instability is not observed FL - Forelimb; HL - Hindlimb (From Song *et al.* J Neurosci Methods 2016;268:117-24).

potential and extent of recovery [Table 1],[135] but data are conflicting. Nakajima and Ikada[90] reported that PEG should be applied for 1 min to avoid overfusion (that leads to cell death) in cell cultures and that at least 10 min are necessary for significant morphological changes to occur indicating that membrane fusion does not materialize instantly on exposure but gradually proceeds with time: optimum molecular weight for fusion occurred at PEG concentrations of 50% w/w (<30% was ineffective) and a molecular weight around 1000. Hoffman *et al.*[53] reported that PEG at a concentration of 75% may be the optimal concentration in cell cultures. Kouhzaei *et al.*[71] showed that the lower PEG's molecular weight, the higher was the ultimate recovery of spinal cord evoked potentials (i.e. PEG 200:49.5% and PEG 2000: 16.3%). Lower molecular weight PEGs caused higher membrane sealing rate (77.8 ± 3.5 for PEG400 [20% w/w] vs. 32.1 ± 6.9 for PEG2000 [20% w/w]). PEG1000 and 2000 showed no significant sealing effects at high concentrations

Box 2: A brief history of PEG fusion in the nervous system.

In the 1970's, polyethylene glycol (PEG) was applied to improve the efficiency of hybrid formation between cells of the immune system and to enlarge the spectrum of monoclonal antibodies which such hybrid lines ("hybridomas") can supply (as per Kohler and Milstein's work in 1975) due to its high efficiency as a fusing agent for fibroblasts[69] and its ability to yield hybrids in cell combinations recalcitrant to *Sendai virus*.[17] O'Lague and Huttner[97] first applied PEG to produce in culture giant multinucleate pheochromocytoma cells - PC12 - cells that expressed various neuronal properties and contained catecholamines. Following this study, Bittner *et al*.[13] extended this observation to axons and employed PEG to repair the cut ends of an invertebrate myelinated central nervous system in the earthworm. PEG-induced fusion rates were as high as 80–100% with an appropriate choice of PEG concentration and molecular mass, tight apposition and careful alignment of the cut ends, and treatment with hypotonic salines containing reduced calcium and increased magnesium.[72] These results should have spurred a flurry of clinically oriented studies that instead never materialized. It was only in recent years that the first clinical application was published by his group.[8] However, recent studies employing Bittner's protocol did not replicate his results: Behaviorally negative studies have been published by independent laboratories (femoral nerve, facial nerve, and facial nerve).[107,15,114] Parenthetically, axonal fusion has been achieved with other methods, namely lasers[136] and electric fields generated by electrical pulses of 10–100 ms duration and 80–200 V amplitude.[128] In 1999, the first application of PEG in a spinal cord model was published by Borgens' group. Shi *et al*.[118] pressed together the ends of completely severed strips of isolated guinea pig thoracic white matter maintained *in vitro* in a double sucrose gap recording chamber and immediately applied polyethylene glycol (PEG; MW: 1400–3500 d, approximately 50% by weight in distilled water) directly to this region through a micropipette; PEG was then removed by aspiration within 2 min. Successful axonal fusion was documented by the immediately restored conduction of compound action potentials (CAPs) through the original transection and by the variable numbers of fused axons in which anatomical continuity was shown by the diffusion of intracellular fluorescent dyes through fused axons. These and further studies led Borgens to test PEG as an IV protectant in a dog model of compression injury,[75] but another later study did not confirm his results.[98] However, PEG has never been injected within the first few hours of SCI, and further studies are certainly warranted. Interestingly, Bittner's group showed that PEG can still fuse severed spinal axons maintained at 6–9°C 1.5 days later, as opposed to 3 h at body temperature[85] pointing in our opinion to a combined hypothermia-PEG study in man.

Sadly, all these studies did not stimulate further independent replications over the years since 1986, and it was only in 2013 with the proposal of the GEMINI spinal cord fusion protocol[18] and worldwide interest in its clinical implications that PEG has gotten a new lease on life.

Box 3: A brief history of propriospinal neurons: Discovered, forgotten, and rediscovered and why they matter.

Starting in the 1940s, functional neurosurgeons started targeting the pyramidal tract in patients afflicted by movement disorders. In 1964, on the basis of his experience with this surgery, US neurosurgeon Bucy *et al*.[16] concluded that "*The pyramidal tract is not essential to useful control of the skeletal musculature. In the absence of the corticospinal fibers, other fiber systems, particularly some multi-neuronal mechanism passing through the mesencephalic tegmentum, are capable of producing useful, well-coordinated, strong, and delicate movements of the extremities*". In 1968, on the basis of extensive studies on monkeys submitted to bilateral corticospinal tract lesions, Lawrence and Kuypers[76] confirmed that brainstem pathways function as the basic system by which the brain exerts control over movement, including erect posture, integrated movements of body and limbs, gait, and use of the extremity and hands. The corticospinal connections act in parallel, with a stronger control of individual movements of the fingers.

They wrote:

"In these animals, following operation, there was an immediate ability to sit…stand, walk, run, and climb…yet…unable to use their extremities, especially their hands, independently of total body movements (yet) they could use them in clinging to the cages and in climbing. After further recovery, …they regained…independent use of their extremities and within 3 weeks could reach accurately with either hand to pick up morsels of food by closure of all fingers in concert…ultimately the animals could fully extend either arm with the wrist slightly dorsiflexed and the fingers semiflexed and abducted."

Thus, primates can perform arm and hand movements (including the dexterous movements of the fingers and precision grip) without a pyramidal tract due to the neural circuits of the propriospinal system alone. In other words, efferent motor control in humans is redundant, with two major motors (sensory) highways feeding into the cord. As explained in the primary text, this observation underpins the GEMINI spinal cord fusion protocol.

This propriospinal meshwork was first mentioned by Edinger[36] at the end of the 19th century and then described in the first half of the 20th century by Sherrington who demonstrated that axons "*springing from the grey matter*" of the spinal cord connect both proximal and distal spinal segments.

He argued that multiple spinal segments communicate with each other to allow complex or "long" motor reflexes. After extensive studies in primates and humans, Laruelle wrote:[21,22]

"*L'association plurisegmentaire est réalisée, non seulement par les voies cordonales connues, mais par un système de fibres intrinsèques de la substance grise, pouvait parcourir plusieurs segments successifs: Elles confèrent une fonction conductrice à la substance grise de la moelle (The plurisegmentary association is brought about not only through the known cordonal pathways but also through a gray-matter-based system of intrinsic fibers, which cover up to several cord segments: These confer conductive properties to the cord gray matter)"*

In the 1940s, David Lloyd[82,82b] provided compelling electrophysiologic evidence that lumbosacral motor pools receive descending inputs that are relayed by propriospinal neurons located in the cervical spinal cord and that reticulospinal and propriospinal fibers form a continuous network stretching from the brainstem through the cord.

After a long neglect, this system has received renewed attention in the 21th century with a focus on recovery from SCI.[9,22,142]

(>50%). Our study[135] found PEG 1400 superior to PEG 4000, but both led to recovery. Wang *et al.*[129] found that 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(poly (ethylene glycol)) 2000] can achieve electrophysiological conduction in isolated spinal cords as effectively as PEG 2000 (50% concentration in Krebs' solution applied for 4 min), but at much lower concentrations than PEG. In any case, PEG has an extremely rapid action. Kim performed cervical laminectomy at C5 in a rat SCI model and then immediately applied PEG-600 or saline. Measurements of motor evoked potential (MEP) found that PEG-treated animals showed an increase in the measurement of MEP's amplitude (mean of 0.081 vs. 0.156 mV) at 1 h after injury.[64]

PEG has been combined with graphene nanofibers that are known to promore axonal regeneration.[65,67,121] and also carry electrical charges. The nanoscale material may be useful for enhancing neuronal signaling by direct contact with the neurons: Kim *et al.*[65] reported near-normal recovery of SSEPs after SCT in rats at 24 h versus none in controls. *In vitro*, nanocomposites composed of 20% w/v PEG and 0.1% w/v multi-walled carbon nanotubes result in high neurite outgrowth and neurite length: electrical stimulation (30 V m-1 DC for 1 h) further significantly enhances this growth up to two-fold.[56]

Another fusogen is chitosan, a nontoxic, biodegradable polycationic polymer with low immunogenicity that has been extensively investigated in various biomedical applications. Topical application of chitosan after complete transection of the guinea pig spinal cord facilitated sealing of damaged neuronal membranes and restored the conduction of nerve impulses through the length of spinal cords *in vivo*.[30]

THE ANATOMICAL BASIS OF SPINAL CORD FUSION

Although experiments show that PEG can refuse severed spinal cord fibers, yet the number is limited (10–15%); in addition, fibers are not matched at the moment of fusion. It can be argued that the reason for its effectiveness is mostly due to PEG neuroprotectant potential of the cord gray matter cellular milieu. In other words, PEG does not actually achieve its goal by refusing a large number of long-projection fibers in the white matter brought together by manipulation of the transected ends of the spinal cord[118] rather it protects the spinal propriospinal matrix that is truly responsible for much of motor and locomotor activities.[21,22]

In mammals, including monkeys and man, there exists a network of interneuronal cells located throughout the rostrocaudal length of the brainstem and spinal cord that conveys motor (and sensory) signals and that embeds and connects the brainstem,

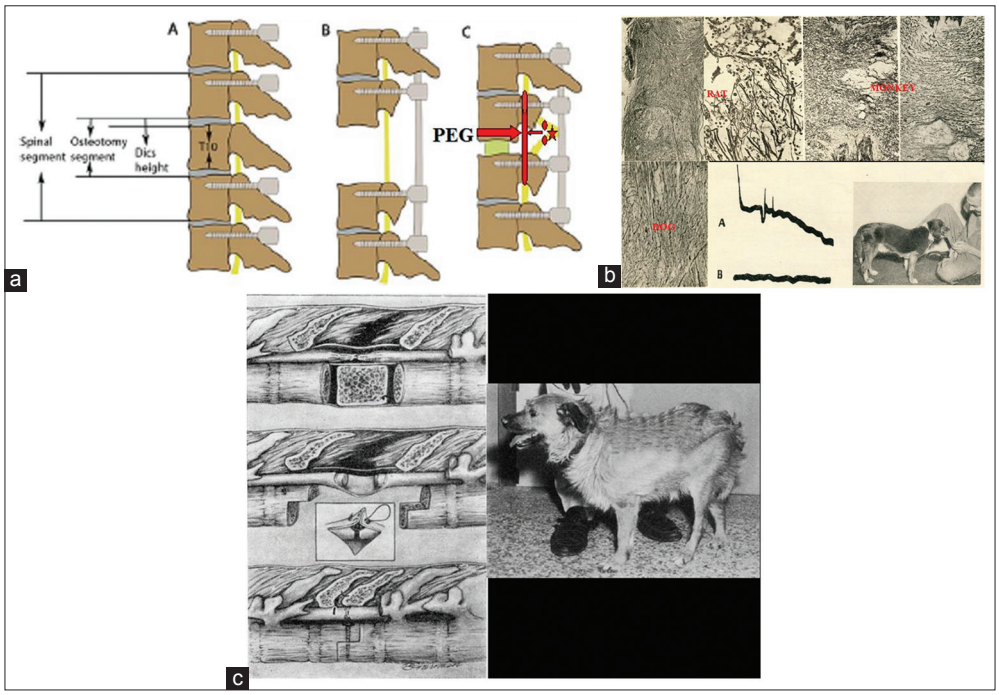

Figure 1: (a) Proposed model of removal of the injured segment (star), transection of the cord above and below (ovoids) and fusion with polyethylene glycol (arrow) along with vertebral shortening and stabilization (adapted from Qiu *et al.*, 2015). (b) Box 1: Freeman discovered that regrowing fibers could be made to grow across the sectional interface in rats, dogs and monkeys and that this translated into electrophysiological transmission and behavioral recovery. (c) Box 1: Since spinal cord transection is not common, Freeman reasoned that he could leverage his technique of spinal cord regeneration in clinical models of spinal cord injury by cleanly cutting the cord above and below the level of injury, removing the injured segment of the cord (2 cm), doing a vertebrectomy and bringing the two fresh ends of the cord together and holding them in place with plasma clot before the dura mater was tightly closed. Dogs could thus be made to rewalk for short distances (the one displayed had almost two thoracic segments removed).

cervical and lumbar central pattern generators [so-called cortico-truncoreticulo-propriospinal system – CTRPS – or Motor Highway 2: Box 3].[21,22] Evidence in humans supports the key role of this system in recovery from SCI.[22]

Spinal fusion is made possible because transection only minimally damages a thin layer of cells belonging to this matrix, allowing the gray matter neuropil to immediately resprout severed axons and dendrites (regenerative sprouting) at the interface of the apposed cords. It should be noted that a sharp transection typically generates <10 Newtons (N: SI unit of force) of force versus approximately 26,000 N experienced during clinical SCI, a 2600 times difference.[122] Iseda *et al.*[58] concluded that "*a single severance, which minimizes damage in the lesion site,…spare(s) nearby cells. On the other hand, a repeated severance inflicts (damage to a) much larger area, which would make it difficult to recruit immature astrocytes in the early postinjur(y) period.*" PEG would protect all cells damaged by the blade. The histological evidence of propriospinal circuits regenerating synaptic connections across the spinal cord transection site is clear-cut.[37,127,133]

An important concern is scarring after SCT. In all published studies, PEG has been applied immediately after SCT. Scarring becomes visible only after about 1 week: given a 1 mm/die regrowth rate, regenerating axons from both cord ends will have penetrated the opposite gray matter well by then (66 mm/h).[94] Nonetheless, there is compelling supporting evidence that the astrocytic scar may actually promote axon regrowth in the early – but not late-stages of SCI.[79,109] PEG does not prevent the formation of a scar and thus does not deprive the regrowth process of the beneficial effects of the early scar past the 1st week.[66,81]

Function will be restored also due to rewiring upstream in the central nervous system (CNS), so long as the mismatch is not extreme. Indeed, recovery from any anatomic disruption of the spinal cord utilizes the entire CNS, namely, cord, brainstem, and brain, in which a massive degree of reorganization (large-scale "rewiring") occurs:[57] mismatches, including those seen in clinical SCI with subsequent recovery, are thus compensated, as in PNS model of fusions.[106]

PAIN AFTER SCT

SCI is followed in up to 40% of cases by so-called cord central pain (CCP).[19,20] CCP is a hugely disabling chronic pain condition that might offset any possible motor benefit of any regenerative treatment. Fortunately, in all animal studies of SCT to date, even at long term, CCP has never been reported. This is a key point. Following cordotomies in man, i.e., section of the spinothalamic (STT) pathways, CCP is seen in up to 20% of the patients. One likely explanation is that CCP is triggered in susceptible individuals by an imbalance between damaged STT and spared lemniscal pathways, which is not the case in SCT, in which both spinothalamic and lemniscal fibers are cut simultaneously.[19,20] However, CCP can follow SCT in man, so this theory does not seem viable. A likely explanation is that acute treatment immediately after SCT somehow quells the pathological cascade from engaging the central pain generator,[19,20] On the other hand, cell grafting for SCI has triggered CCP in more than half of the patients in a study.[68]

CCP is generally accompanied by hyperactivity in the TRPS pathway, which can be quelled by extensive neurosurgical destruction thereof at both brainstem and cord levels: pain is controlled to a major extent.[19,20] Given the model proposed in this review, extirpation of the damaged cord segment followed by fusion might be able to control CCP.

CLINICAL TRANSLATION

Experimental evidence [Tables 1 and 2] make it clear that PEG is most effective when applied locally and acutely on lesioning. This can be tapped with different approaches, all based on the removal of the injured segment of the cord.

Gemini

As discussed, Walter Freeman suggested the severance-reapposition model for chronic SCI; he removed the damaged segment of the cord in dogs creating a gap, performed a complete *en bloc* vertebrectomy thus shortening the spine, brought the two fresh cord stumps in contact with fresh plasma and sutured the dura tightly: walking animals resulted after several months. He observed direct electrophysiological conductance across the apposed stumps and provided histological evidence of axonal regeneration across the sectional interface [Box 1].[39-41,21,23,51,52] Spine-shortening vertebral osteotomy (a.k.a. vertebral column resection), which shortens the spinal column, is a surgical technique for correcting severe spinal deformities, treating congenital spinal anomalies, such as cord tethering, traumatic spine dislocations, and spine tumors at both cervical and thoracolumbar levels.[5,54,87,101,123] In the proposed GEMINI model,[21,22] section of the damaged segment of the cord is performed at the moment of removing the vertebral body; the two ends will need further trimming so that no undue pressure is exerted on either stump by pressure vectors (too much pressure would lead to squeezing and local ischemia, jeopardizing the result). PEG is applied at this moment. Notice that the vertebra has been removed and stabilization carried out simultaneously [Figure 1a].[21-23] Another way to stabilize the fusion interface has been proposed recently: Brazda *et al.*[14] kept the two spinal cord ends in apposition by a microconnector system incorporating a microchannel system, through which PEG was infused through a minipump. This allowed a tension-free, precise apposition of sharply transected nerve spinal cord stumps, as required by GEMINI. The spinal cord tissue staid in place within this device after the tissue opposition maintained by this vacuum system was released. The minimal, gradual stretch to the axons actually stimulated regrowth. However, until biodegradable connectors are built, this technology remains unviable in man.

Hydrogelation of the GAP

PEG can be cross-linked to form porous hydrogels, which can serve as biocompatible matrices that can closely mimic the ECM. This

Box 4: The first cord graft for SCI in man.

In 1906, Shirres[119] reported on the first such case, JC, sailor aged 48, a patient whose cord had been traumatically transected:
"Dr. Armstrong asked me whether I thought it would be of any use to transplant a dog's cord between the ends of the divided cord of our patient. My reply was that I did not know what would occur but did not think any serious results were likely to follow and it might be worthy of a trial. He decided to have this operation carried out. A large dog was obtained, placed under chloroform, and an operation to expose the cord was carefully done under the most strict asepsis by two assistants, Dr. Barlow and Dr. Campbell. While this was being done our patient was put under chloroform and placed on the table. An incision was made over the seat of the old lesion, the dura mater opened and the cord exposed. At this time we found that a separation now existed between the two ends of the cord, about one and a half inch in extent. With a mild faradic current the anterior and posterior roots in the lower segment of the cord were stimulated, and a faint response took place in the muscles. The dog's cord to the extent of three inches was laid alongside the upper and lower segments of the patient's, a few fine stitches united the pia-arachnoid of the one to the other, the dura mater was closed, the wound sutured, a plaster jacket applied and the patient taken back to the ward. He made a perfect recovery from the operation, the temperature on no occasion going over 100. A month passed without any apparent change. Fortunately, I had another group of students who had attended my voluntary Christmas Vacation Course. I was able to instill in them an interest in the case and was thereby able again to obtain help in giving the electricity and massage that I could not otherwise have done. The 5th week after the operation the patient was conscious of flatus in the lower quadrant of the abdomen. This he had never experienced before. 6 days following this he became conscious of the passage of the catheter, when routine lavage of the bladder was being carried out, and 10 days later he was able to inform the orderly that his bowels were about to move, and could tell when faecal matter passed the rectum. On this date, for the 1st time, he complained of subjective sensations of pins and needles in the right foot, and a week later of the same symptoms in the left foot. 2 months after the operation he described vividly and with all assurance subjective disturbances in both feet extending up to the knees. The passage of the catheter and the evacuation of the bowels were much more clearly felt. At first, we were inclined to think that this must be purely imagination, but when one heard the patient describe the condition with such exactitude, its coming and going, one began to think otherwise. Another reason for our coming to the belief that those feelings were real was that after the first operation the patient had as much care as after the second, and his desire and hope of recovery was just as keen if not more so than after the second, yet he never by any means gave the suggestion that such symptoms as above described were at any time present. Little alteration, progress or otherwise, from the above was noted until about the 18th day after the operation, when it was detected for the 1st time that with percussion of the pleximoter on the muscles of the flexor aspect of the thigh and leg, the presence of a certain amount of tone was noticed by the contraction of the muscle. Neither at this time nor at any time since the accident had voluntary movement or the return of objective sensory symptoms taken place. The reflexes, superficial and deep were still absent.
From day 16, the patient's conditions degenerated and died 2 weeks later. At autopsy,
"The cord was carefully removed and placed in Muller fluid to harden. 6 weeks later I opened up the dura mater and found lying between the two ends of the cord, where the injury had occurred, a diffluent mass. Sections of the cord above and below the lesion were put aside for Pal-Weigert stain, and the dura mater with its adherent mass between the ends of the cord was likewise prepared for Pal-Weigert stain. The sections of the upper segment revealed the typical ascending degenerations in the fields of Goll and Burdach, the direct cerebellar, and Gower's tracts. The sections below showed definite degenerations in the crossed and direct pyramidal tracts. The dura with its adherent substances which lay between the ends of the cord, after being stained by the Pal-Weigert method, showed a mass of minute myelin sheaths of nerve fibre which you may see by looking through the microscope placed before you. These fibers can be seen lying closely adherent to the dura mater and when traced upward and downward through the different sections, unite with the segments of the cord above and below, demonstrating the fact that regeneration of the axons of the spinal neurons had taken place, to a limited extent. Pal-Weigert stain, as you know, stains only the myelin sheaths. At the time of the operation, the dura mater between the two segments was perfectly clear of nerve fibers to the naked eye. I do not for a moment suggest that the dog's cord retained its vitality after it had been removed and placed inside the dural sheath, nor would I like to suggest that it was the dog's cord started the regeneration. I only want to speak here of the following facts, which you will see demonstrated under the microscope, that the nerve fibers are present and that they unite the two segments of the cord. Unfortunately, I did not place the cord in formalin or alcohol and, therefore, was unable to make a study of the lower segment by the Nissl method. From the sections, you will see that this lower segment is comparatively speaking, in a fairly healthy condition. Sections through the cauda equina showed very little change; indeed, 90%, of the nerve fibers making up this structure were to all intents and purposes normal by the Pal-Weigert stain. I had the pleasure of showing the specimens at a meeting of the Lister Club at McGill University, where the pathologists without hesitation assented to the view that regeneration had taken place."
He concluded:
"*The success, in this case, I think, was largely due to the patient having been so assiduously treated by electricity, the prevention of contractures and the frequent movement and massage of the extremities.*

suggests another possibility that does not require a vertebrectomy: removing half of the damaged cord, up to its border with rostral and caudal healthy tissue and filling the void with a PEG hydrogel. PEG hydrogels have high water content and porosity, which make them behave like aqueous solutions at a microscopic scale while being macroscopically solid. In an easily tailorable process, these can be optimized by adding different reactive moieties to both ends of the PEG chain. Mosley *et al.*[89] determined that a Young's modulus of 907 Pa allows for the longest axonal extensions, which closely abide with the Young's modulus of the brain and spinal cord.[99] In any case, pure PEG *per se* is enough to warrant clinical trials without more expensive modifications. Injectable PEG, by *in situ* gelling, can conform geometrically to the defect without requiring a pre-gelled patient-specific hydrogel or causing additional

excision of healthy tissue.[70,83] Although PEG hydrogels can be used as supporting substrates, for example, of mesenchymal stem cells, inducing cell migration, proliferation, and differentiation,[50] this strategy has not been found to be synergistic with PEG in one rodent study.[96] Regenerating propriospinal fibers would course through this hydrogel, which has been proven to lead to recovery after many months.[37] Certainly, the use of PEG alone cannot completely mimic the three-dimensional porous structure of the spinal cord and would allow the upper and lower fiber bundles to grow in mismatched or even misplaced channels or pores, which is not the case with Freeman's appositional model. Moreover, this approach would need months for recovery and outcomes would be partial, as when the two stumps have been joined with different strategies in chronic SCI patients.[46,125] However, microspheres loaded with neurotrophins (e.g., BDNF and GDNF) could be embedded for slow release to accelerate this regrowth.[73]

Fusion-supported cord grafting

The possibility of implanting a segment of healthy cord from an organ donor must be also entertained [Box 4].

In this case, PEG would neuroprotect the tissue until vascularization from the healthy ends of the patient would feed the graft. Biomaterials can be effectively used for promoting and guiding blood vessel formation.[7,48] PEG hydrogels support the formation of vascularized tissue in vivo in a pore size dependent manner.[29] and PEG has been shown to promote angiogenesis in an SCI model.[37]

PEG proxies

As mentioned, another effective fusogen is chitosan. Rao *et al.*[104] found that NT3-loaded chitosan, when inserted into a 1-cm gap of hemisectioned and excised adult rhesus monkey thoracic spinal cord, elicited robust axonal regeneration: in particular, motor axons in the corticospinal tract not only entered the injury site within the biomaterial but also grew across the 1-cm-long lesion area and into the distal spinal cord, accompanied by motor and sensory functional recovery. Similar data with chitosan scaffolds have been reported in rodents.[92,140]

A combination of both chitosan and PEG in hydrogels promise even better results.[61,88] Blends of photocrosslinkable 4-azidobenzoic acid-modified chitosan (Az-C) and PEG form a semi-interpenetrating network (semi-IPN), where PEG interpenetrates the Az-C network and reinforces it. Nerves anastomosed with an Az-C/PEG gel tolerate a higher force than those with fibrin glue; Az-C/PEG gels are compatible with nerve tissues and cells. In addition, Az-C/PEG gels release PEG over a prolonged period, providing sustained delivery of PEG.[2]

Electrical stimulation

As originally proposed,[21] the entire fusion process can be accelerated by electrical stimulation. Progress has been made by electrical stimulation of the cord combined with intensive, months-long rehabilitation, although full independent recovery has not been achieved.[3,45,60] In GEMINI, electricity would combine central (e.g., rTMS) and/or spinal cord stimulation and/or peripheral stimulation (e.g., TENS), along with motor training, to accelerate regrowth of fibers from the TRPS network across the fusion interface.[24,130] As noted [Box 4], it was Shirres who first emphasized the ability of electricity to stimulate spinal cord regeneration.

CONCLUSION

Removing the chronically injured segment of a cord, followed by spinal shortening and PEG fusion of the healthy ends (GEMINI protocol) has the potential to restore motor function in a substantial number of chronically paralyzed (ASIA A) patients for whom no cure is available.

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Conflicts of interest

There are no conflicts of interest.

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