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*Original Article*

# PEG-chitosan (Neuro-PEG) induced restoration of motor function after complete transection of the dorsal spinal cord in swine. A pilot study

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## ABSTRACT

**Background:** Spinal cord injury (SCI) remains an unmet medical need. Recently, fusogens, such as polyethylene glycol (PEG), have been proven effective in restoring sensorimotor function after complete transection of the spinal cord at different levels and in different species. Here, we report on the use of a PEG-chitosan combo in a different animal model (swine).

**Methods:** Five Hungarian Mangalica pigs were subjected to complete transection of the thoracic cord (T7-9). Three animals were treated with locally injected PEG-chitosan (Neuro-PEG) gel; two acted as controls. PEG-600 was also injected intra- and post-operatively intravenously. Animals were submitted to rehabilitation, including electrical myostimulation. Results were assessed after 60 days using the Individual Limb Motor Score, the Porcine Thoracic Spinal Cord Injured Behavioral Scale, and the modified motor Basso, Beattie, and Bresnahan scale; sensory and sphincter functions were also assessed. Animals underwent *in vivo* spinal cord tracing with DiI. Immunofluorescence histology included NF-200, DAPI, and a fluorochrome-conjugated secondary antibody.

**Results:** Starting on postoperative day (POD) 2, neuro-PEG-treated animals evinced the first signs of recovery, and on POD 60, they could all support their weight and were mobile. Controls never recovered any useful function. Fluorescence microscopy in the experimental group revealed axons passing through the site of injury, while degenerative post-traumatic changes were noted in controls.

**Conclusion:** Neuro-PEG affords sensorimotor recovery after complete spinal cord transection. This opens the door to human experimentation, including trials of spinal cord transplantation.

**Keywords:** Chitosan, Fusogens, Fusogen-sealants, Neuro-PEG, Polyethylene glycol, PEG-chitosan, Spinal cord injury

## INTRODUCTION

Spinal cord injury (SCI) followed by permanent paralysis represents an unmet clinical challenge.[17] Despite touted advances in neuromodulation of severely disabled SCI patients,[9,16]

no patient has so far recovered naturalistic motor function after full spinal cord transection. Moreover, only the motor axis is targeted.

Starting in 2013,[4] fusogens, that is, substances that can restore the integrity of severed nerve fibers, have been tested in animal models of complete transection of the cervical and dorsal spinal cord, with the recovery of ambulation.[10,18] Further to this, several translational approaches of such substances (e.g., polyethylene glycol [PEG] and chitosan) have been put forth for deployment in paralyzed SCI subjects, including spinal cord transplantation.[3]

In view of these results, our team developed a conjugate based on PEG and chitosan (PEG-chitosan or NeuroPEG), which showed its effectiveness in rodent and lagomorph models.[11-14] Here, we report for the first time the results of the Neuro-PEG application in swine.

## MATERIALS AND METHODS

We used Chitosan with a molecular weight of 15 kDa (Merck, Germany), stabilized by photo-crosslinking, in a homogeneous mixture with PEG-600, (Merck, Germany), at a concentration of 20 mg/mL. The resulting mixture was sterilized and stored in sealed tubes at a temperature of 4°C (Neuro-PEG).

After obtaining institutional approval from the Local Ethics Committee, female pigs of the Hungarian Mangalitsa breed (m = 20.0 ± 2.0 kg, $n$ = 5) were used (three experimental and two controls). All animals were treated in accordance with the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes. The animals were anesthetized with a mixture of Zoletil (Virbac, France) and Xylazine (Alfasan Int., Netherlands) intramuscularly, followed by propofol (Propofol-Kabi - Fresenius Kabi, Germany) through a central venous catheter. The animals were then paralyzed and intubated (air-oxygen mixture); a urethral urinary catheter was inserted.

### Surgery

A T7-9 extended laminectomy was performed in standard fashion. The T7-9 segment was then stabilized with screws and rods: dorsal traction was applied. After that, a Smith-Peterson subtraction spondylotomy was performed. Ice slurry from a frozen isotonic sodium chloride solution ($t$ = 0±0.5°C) was placed on the dural sac for 1 min. The dura mater was then opened longitudinally and fixed laterally. A flexible spatula was placed under the spinal cord to protect the dura, and the cord was fully transected with a No. 11 scalpel. The resulting diastatic space was flushed with 2 mL of neuro-PEG. In parallel, an intravenous drip infusion of 50 mL of PEG 600 solution was performed. Control animals ($n$ = 2) were injected with saline. Water-tight sutures were applied to the dura, and the suture itself was covered with fatty tissue. After careful hemostasis of the wound, a vacuum drain was installed. The wound was sutured in layers and covered with an aerosol bandage [Figure 1].

### Postoperative rehabilitation

Post-operatively, antibiotics and analgesics were administered. A day-night regime was instituted with free access to water and regulated nutrition. The premises were air-conditioned, and constant temperature was maintained. The animals underwent daily massage of the hind limbs to improve trophism and prevent bedsores. Daily electromyostimulation was also used on the limbs (3–13 Hz, PW 0.1–30 ms, 20 min/limb, bid) (experimental electromyostimulator "TiT," LLC «TiT», Russia). Subcutaneous injections of neostigmine methyl sulfate (18 mcg/kg twice daily) and intramuscular injections of trypsin (10 mg once daily) were given for 15 days. For seven days, the three experimental animals were injected with IV PEG 600 (50 mL of a 25% solution of standard saline), qid. During rehabilitation, animals were placed on all four limbs with pelvic support. The urethral catheter was only removed after restoration of urination, as

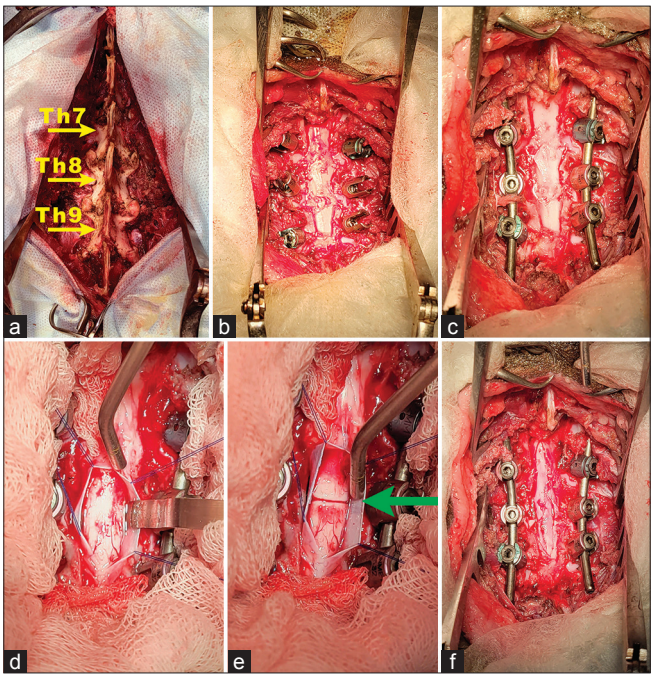


**Figure 1:** Operative steps. (a) Skeletonization of the vertebral arches of T7, T8, and T9 (Yellow arrows); (b) laminectomy and access to the spinal cord. Pedicle screws are installed; (c) the longitudinal rods are installed with the traction of the screws; (d) the dura mater is dissected, and lifted on thread-holders; a spatula is placed under the spinal cord; (e) axial section of the spinal cord (green arrow); and (f) dura closure performed [cranial end on top].

assessed every three days after test removal of the catheter for six hours. Defecation deficits were countered with enemas [Figure 2 for an overview of the experiment].

### Neurologic assessment

Neurologic assessment (motor) was carried out using the Individual Limb Motor Score [Figure 3], the Porcine Thoracic Spinal Cord Injured Behavioral Scale [Figure 4], and the Modified Motor Performance Score as per Basso, Bresnahan [Figure 5].[1,8,15] Scoring on all three scales was conducted with animals placed in a 6-meter corridor with a soft surface. Needling of the hind limbs was employed to test for the presence of nociception. Pelvic functions were evaluated based on the presence and volume of diuresis and defecation, and intestinal motility was assessed by auscultation. A blinded third party made all assessments.

### Immunofluorescence

After induction of anesthesia with Zoletil, on post-operative day (POD) 50, microinjections of 5 µL of a 2% solution of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) (Abcam, UK) in dimethyl sulfoxide - (DMSO, Merck, Germany), were performed into the spinal cord 3 cm above the injury site, using a Hamilton syringe for 5 min. On day 61, all animals were euthanized with an overdose of Zoletil and Xylazine; a 10-cm section of the

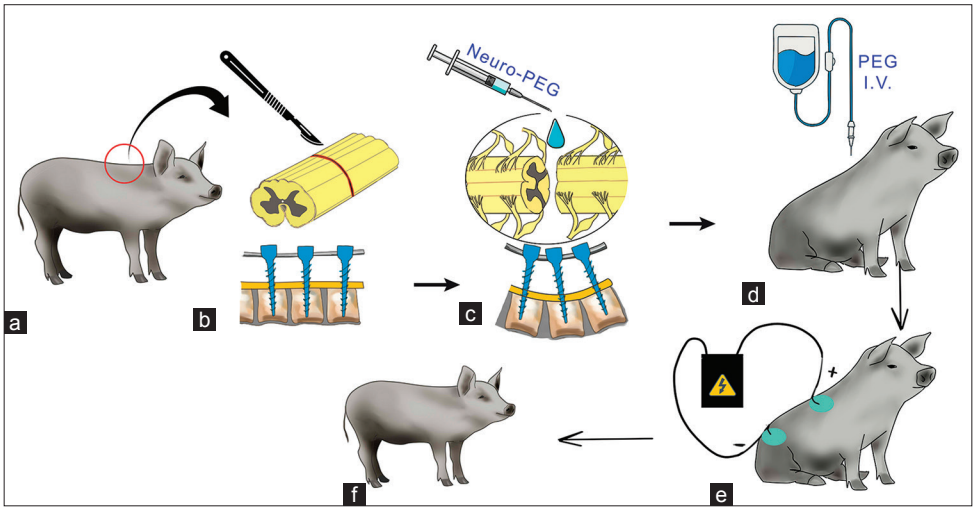


**Figure 2:** Design of the experiment. (a and b) transection of the spinal cord at the thoracic level; installation of pedicle screws; (c) irrigation of the sectional surfaces of the spinal cord stumps with Neuro-PEG; vertebral stabilization with pedicle screws; (d) IV infusion of PEG in the postoperative period; (e) neurorehabilitation, including electrical myostimulation; and (f) early verticalization of animals after surgery.

| Individual Limb Motor Scale (ILMS) | |
|---|---|
| **Score** | **Description** |
| 1 | Flaccid, inactive limb |
| 2 | Weak and irregular limb movement, no weight bearing |
| 3 | Robust and regular movement in one or more joints, abnormal joint angles and limb positioning, no weight bearing |
| 4 | Normal positioning of the limb, partial weight support, abnormal joint angles |
| 5 | No visible impairment for joint flexion and extension, partial weight support |
| 6 | Complete body weight support for standing but not for stepping |
| 7 | Apparently normal stepping with mistakes in limb coordination |
| 8 | Apparently normal use of the limb |
| Total score(MAX) 8 | |

**Figure 3:** Individual Limb Motor (scale).

| Porcine Thoracic Injury Behavior Scale (PTIBS) | |
|---|---|
| **Description** | **Score** |
| No active hindlimb movements, with rump and knees on the ground. | 1 |
| Active hindlimb movements, with rump and knees on the ground. | 2 |
| Active hindlimb movements, with "weight-bearing extensions" that lift the rump and knee transiently off the ground (hip joints are flexed but knee joints flexed and extended). | 3 |
| Active rhythmic hindlimb crawling with at least 3 reciprocating gait cycles (Crawling: L-R-L-R-L-R). Rump off the ground constantly and transient "weight-bearing extensions". | 4 |
| The animal can take at least two steps (and up to six steps) with the rump and knee constantly off the ground during the steps. The knees do not fully extend. Hoof placement is a combination of dorsal and plantar. Balance while stepping is impaired. | 5 |
| The animal can take more than six steps with the rump and knee constantly off the ground. The knees do not fully extend. Hoof placement is a combination of dorsal and _ plantar. Balance while stepping is impaired. | 6 |
| The animal can take at least two steps (and up to six steps) with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired. | 7 |
| The animal can take more than six steps with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired. | 8 |
| The animal can take more than six steps with the knees fully extended. Hoof placement is planter. Trunk imbalanced as the animal steps (walks). | 9 |
| The animal demonstrates grossly normal ambulation, with normal balance. | 10 |

**Figure 4:** Pig with Thoracic Spinal Cord Injury Behavioral Scale. L: Left, R: Right.

spinal cord, including the area of injury, was removed and immediately fixed in a 4% solution of paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) with pH 7.4. The samples were cryoprotected in 20% sucrose at 4 °C for 48 h, and the frozen samples were cut into longitudinal horizontal sections 30–35 μm thick using a cryotome. For permeabilization, slides with sections were immersed in Tween-20 0.3% (Abcam, UK) for 20 min. Blocking of nonspecific binding was carried out in 1% FGBA (gelatin solution ×10 Fish Gelatin Blocking Agent, Biotium, USA). Afterwards, a dilution of monoclonal mouse primary antibodies against neurofilament 200 (NF-200) (monoclonal mouse antibodies Anti-200 kD, NF-200 – Neuronal Marker, Abcam, UK) was prepared in 1% FGBA, and slides were incubated in this solution for one hour at room temperature. Dilutions of secondary antibodies conjugated with Alexa Fluor 488 fluorochrome (Rat monoclonal [SB84a] Anti-Mouse IgG2a heavy chain secondary antibodies [Alexa Fluor® 488], Abcam, UK) were also prepared with 1% FGBA. Incubation with secondary antibodies was carried out in the dark for one hour. Cell nuclei were stained with a 300 nM solution of 4',6-diamidino-2-phenylindole – DAPI (Abcam, UK) in PBS. The slides were allowed to air-dry, and a drop of mounting medium was added. The specificity of staining was assessed by excluding primary antibodies or by incubation with FGBA. Fluorescence microscopy was performed using an AxioScope A1 microscope (Zeiss, Germany), a BioTek Cytation-1 multimode cell imaging module (Agilent Technologies BioTek, USA), and BioTek Gen5 software (Agilent Technologies BioTek, USA) with appropriate filters. The sections were studied and micrographed. Photo processing was carried out using ImageJ software (NIH, USA).

### Statistical analysis

One-way analysis of variance and Spearman correlation tests were used to assess for significance for all data sets (threshold for significance: $P \leq 0.01$)

## RESULTS

### Neurologic recovery

Post-operatively, all animals evinced paraplegia, anesthesia of the lower half of the body and sphincter incontinence. Coprostasis and urinary retention were equally pronounced up to post-operative day (POD) 2 in both groups. Subsequently, controls showed no signs of recovery over 60 days. On the other hand, experimental animals showed signs of recovery from POD 1. One animal evinced clear movements of the hind paws on the horizontal plane (weak pulling of the paws under active abduction by the experimenter). On POD 2, it reacted to a needle prick in three out of six control points on the skin of the hind limbs, and by POD 4, sphincter (urinary and bowel) control was restored. The other two animals had their catheters removed on POD 10. On POD 6, one treated animal showed active

| Modified Basso, Beattie, Bresnahan Motor Activity Assessment Scale in Experimental Animals with Spinal Injury, Including Sensitivity Descriptions and Assessment of Pelvic Functions (mBBB) | | | |
|---|---|---|---|
| **Description** | **Score** | **Description** | **Score** |
| **MOTOR ACTIVITY** | | | |
| No noticeable movement of the hind limbs; | 0 | Periodic plantar steps based on weight; no coordination of the hind limbs; | 10 |
| Slight movement of one or two joints within the plane; | 1 | Frequent or consistent weight-supported plantar steps and lack of hindquarter coordination; | 11 |
| Extension movement in one joint, or heavy movement in one joint and little movement in the other joint within plane | 2 | Frequent or consistent weight-supported plantar steps and occasional hindquarter coordination; | 12 |
| Extension movement of two joints within plane; | 3 | Frequent or successive weightsupported plantar steps and frequent hindquarter coordination; | 13 |
| Easy movement of all three joints of the hind limbs within the plane; | 4 | Successive weight-supported plantar steps, constant coordination of the hind limbs, frequent plantar steps; | 14 |
| Small movements in two joints and extensive movements in the third joint within plane. | 5 | Constant plantar step and constant coordination of the hind limb and the predominant position parallel to the body. | 15 |
| Extensive movement in two joints and slight movement in the third. | 6 | Constant plantar step and constant coordination of the hind limb; the predominant position of the limbs is parallel. | 16 |
| Extensive movements of all three joints in the hind limbs. | 7 | Constant plantar step and constant coordination of the hind limb, the predominant position of the limbs is parallel or inward at initial contact and at break-away. Trunk instability present. | 17 |
| Plantar placement of hindlimb without weight support. | 8 | Constant plantar stride and constant hind limb coordination; the predominant position of the legs is parallel or inward at initial contact and at separation. No body instability observed. | 18 |
| Weight-supported plantar placement of the hindlimb only when stationary, or occasional, frequent or constant dorsal weight-supported striding and no plantar stride. | 9 | | |
| **SENSITIVITY** | | | |
| Lack of response to pain stimulus at 4 different points. | 0 | Attempt to jerk the limb. | 2 |
| Response to pain stimulus and/or an attempt to remove the stimulus or get away from it, while without movement in the limb. | 1 | Active resistance in response to the stimulus | 3 |
| **PELVIC FUNCTIONS** | | | |
| Lack of control over pelvic functions. Spastic disorder. Inability to independently defecate and urinate. Intestinal peristalsis is not auscultated or extremely sluggish, coprostasis. Bladder emptying does not occur. | 0 | Inconsistent control of pelvic functions or retention of urination with a preserved bowel movement or retention of bowel movements with a preserved urination. Periodically needs to divert urine. Intestinal peristalsis is sluggish but regular. | 1 |
| Permanent control over pelvic functions. No delay in defecation and urination. | | | 2 |
| **MAX 23** | | | |

**Figure 5:** Basso, Beattie, Bresnahan modified motor activity rating scale.

attempts to stand upright on its hind limbs. By POD 6, the strength in the hind limbs of two animals was sufficient to push off and counter pressure from the experimenter's hand; one animal independently made active movements with its hind limbs but without attempts to stand. On POD 9, one animal could stand up freely and attempt to walk using its hind limbs [Figure 6]. By POD 10, all three treated animals showed active movements in the hind limbs and attempted to stand without support. Nociception was re-established. By POD 14, two treated animals could independently stand upright, supporting themselves on their hind limbs. The animals could move independently using their hind limbs, but the movements were coarse, and there was pronounced instability when walking. By POD 28, the animals retained moderate paraparesis, but the range of movements increased significantly. Walking

**Figure 6:** Recovery of motor function. 1st row from top: Postoperative day (POD) 6. The animal makes active movements with its hind limbs but without attempts to stand upright. 2nd row from top: POD 6. In one treated animal, attempts to stand upright using the hind limbs were noted. 3rd row from top: POD 9. One treated animal showed the ability to stand upright freely and attempted to walk using its hind limbs. 4th row from top: by POD 21, the animal stands confidently on all limbs, with full support; the range of motion has increased significantly; some instability when walking is visible. 5th row from top: On POD 55, the animal walked independently in the enclosure on all four limbs.

instability was observed, and sensory function was seen at 2–3 cutaneous points. By POD 60, two experimental animals moved independently around the enclosure and used all limbs when walking; 3-point sensory recovery was clear-cut, and the animals exercised full control over pelvic functions [Figures 6 and 7 and Video 1 at POD9]. The third animal recovered substantial levels of motor function and could support its weight on all four limbs, but walked only when prompted by the experimenter and spent most of the time sitting.

All results were statistically significant on all applied scales at POD 60 ($P$ = 0.001; Spearman Correlation Coefficient: 0.726).

### Immunofluorescent staining

In controls, the site of transection was clearly visible, with axonal loss in areas undergoing Wallerian degeneration. A decrease in the amount of NF-200 in the caudal segment of the spinal cord was noted as a sign of axonal Wallerian degeneration. In the area of injury, loose tissue was observed devoid of NF-200 staining; an area of glial degeneration and scarring diffusely stained for DAPI, a nuclear dye [Figure 8], at the level of the diastasis. In treated animals, NF-200 staining was visible at the level of the transection with unevenly located neuron bodies and stained dendrites and axons. Despite the presence of cysts in the gray matter, the white matter was crisscrossed with twisted and thickened axons [Figure 9]. Dil tracing in treated animals only evinced the distribution of the dye through the site of injury. Axons crossing the intersection site are visible [Figure 10].

## DISCUSSION

In this study, we show that fusogens can reverse spinal paralysis after complete transection in a large animal model. Although the study is small, the results are clear-cut.

The previous studies of PEG application in a similar experimental scenario were highly positive,[10,18] including

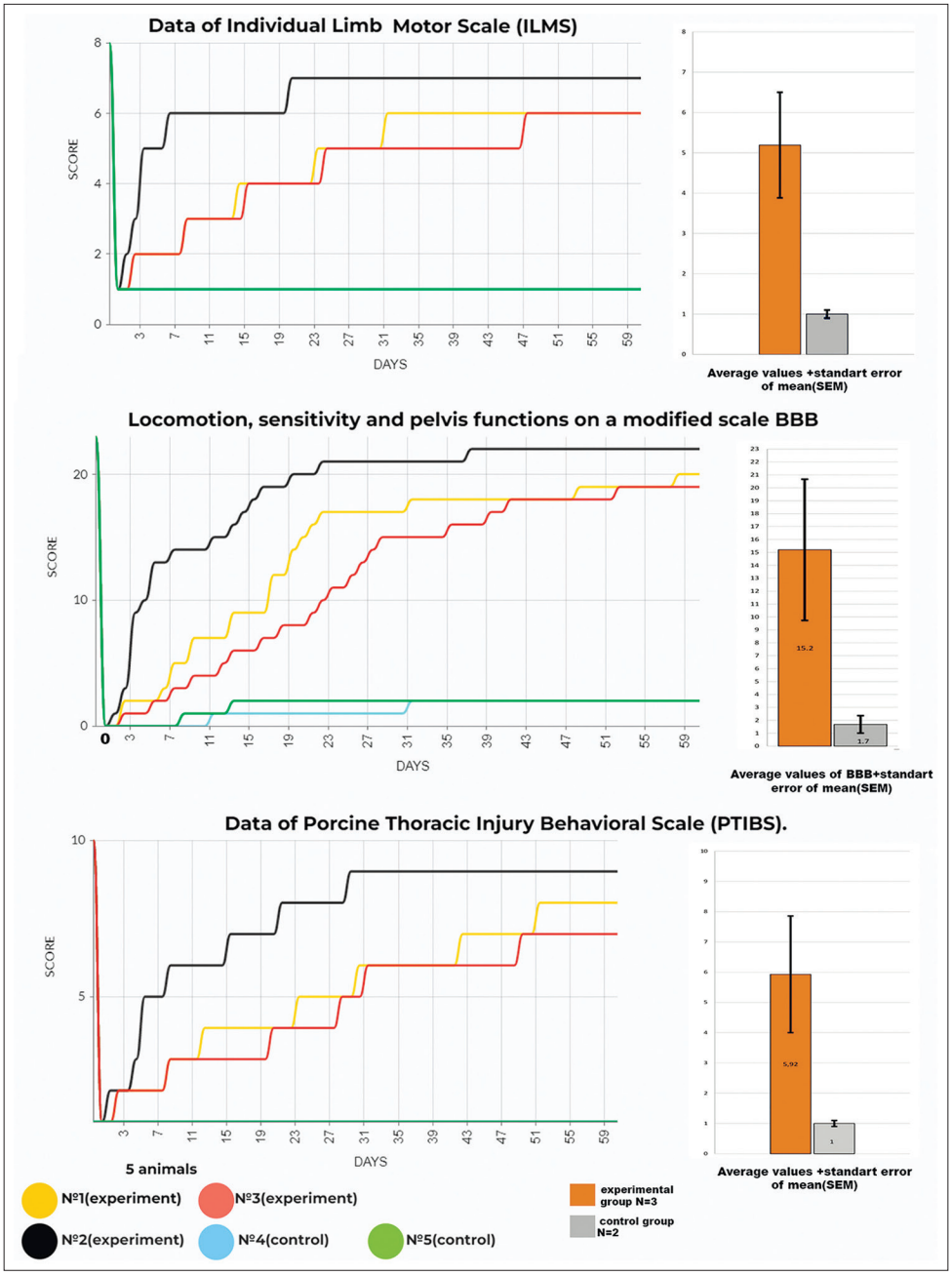


**Figure 7:** Motor activity scores of Individual Limb Motor Score, modified Basso, Beattie, and Bresnahan (BBB), and Porcine Thoracic Spinal Cord Injured Behavioral Scale (No. 1–3 – experimental animals, No. 4 and 5 – controls)—right side: bar charts of means. Data in graphs are shown as means ± standard error of the mean.

in monkeys. However, we feel that our results – pending confirmation in a larger sample – are actually superior. For instance, dogs submitted to full spinal cord transection recovered ambulation, but sphincter control recovery was poor.[10] This is likely due to the superior effect of combining PEG and chitosan, two fusogens with different chemical profiles.[4,19,20] However, similar to the previous studies,[8] behavioral recovery was fast, that is, within 48 h. Both previous studies and ours confirm that PEG600 achieves the best results.[19] We also notice a

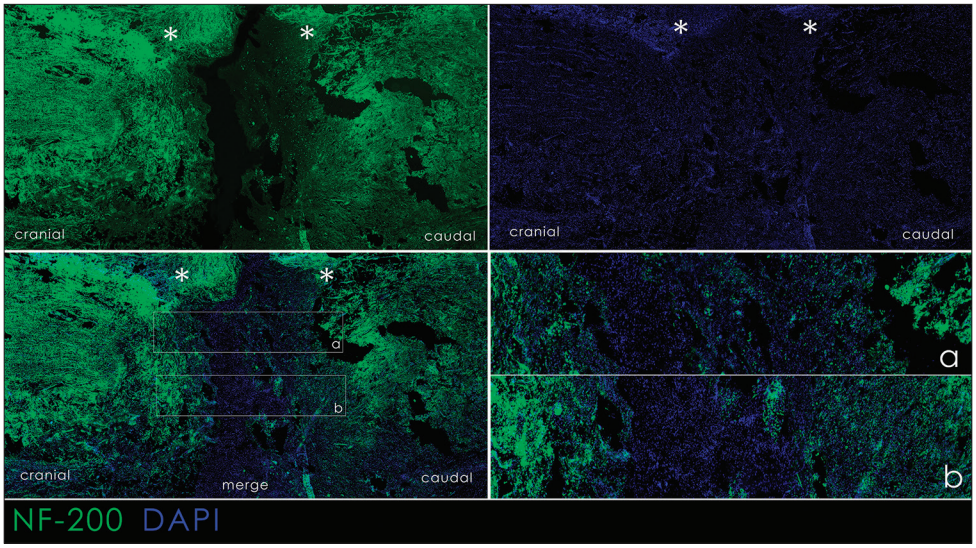


**Figure 8:** Immunofluorescence of the spinal cord of control animals. DAPI staining; staining for neurofilament-200; combination of DAPI+NF-200; (a and b) area of transection. The horizontal section; cranial end are on the left. Asterisks indicate the distal and proximal ends of the spinal cord.

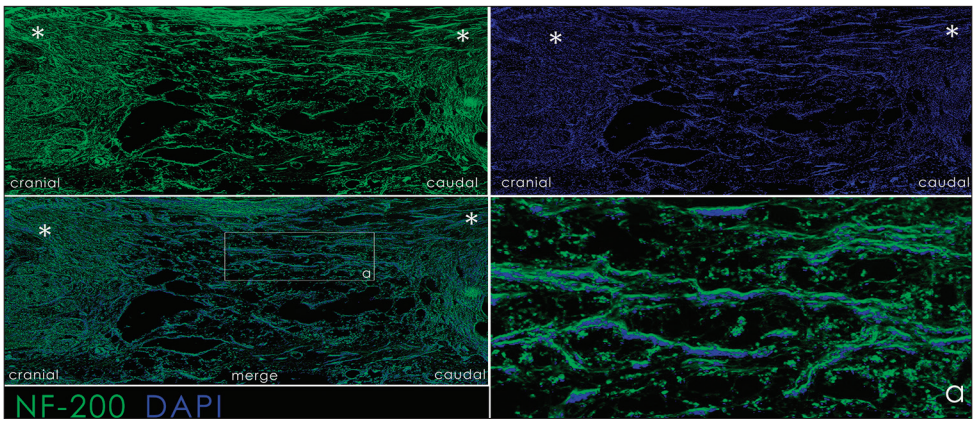


**Figure 9:** Immunofluorescence histology of the spinal cord of experimental animals. DAPI staining and staining for neurofilament-200; a combination of DAPI+NF-200; a: area of transection. The horizontal section; cranial end are on the left. Asterisks indicate the distal and proximal ends of the spinal cord.

possible contribution from IV PEG, as discussed in the literature.[4]

Fusogens are part of the GEMINI spinal cord fusion protocol,[1-3] the engine of head,[4] brain,[6,7] and spinal cord transplantation.[3] In particular, our protocol appears well suited for deployment in the context of spinal cord transplantation as a potential cure for spinal paralysis. Once the damaged segment of the cord is removed, an equally sized segment from a brain-dead organ donor is harvested and installed into the gap, and neuro-PEG is deployed under immunosuppression.

We notice that our approach includes not only immediate local application of a fusogenic mixture but also intravenous injection of PEG over three days, plus local cord cooling as an adjunct neuroprotective measure. To keep the spinal stumps apposed for fusion and avoid displacements, we employed vertebral stabilization for the first time in fusogen SCI studies. Finally, electrical muscular stimulation, along with massage and motivational prompts, complement the method—the contribution of all these components on their own remains to be quantified.

**Video 1:** Postoperative day (POD) 9. One treated animal showed the ability to freely stand upright and attempt to walk using its hind limbs. When walking, instability and ataxia persist. The pig in the video has a urethral catheter reinserted to monitor complete bladder emptying. Sufficient control of pelvic functions was restored in this animal by POD 4.

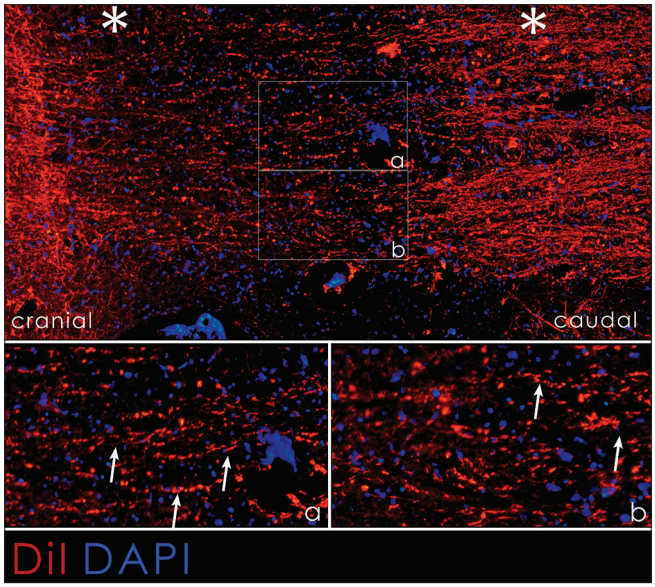


**Figure 10:** DiI immunofluorescence histology of the spinal cord of treated animals. Combination of DAPI+DiI; (a and b) area of spinal cord injury. The horizontal section; cranial end are on the left. Asterisks indicate the distal and proximal ends of the spinal cord. White arrows indicate the axons crossing the intersection site.

## CONCLUSION

We present the results in a large animal model of the use of our proprietary fusogenic PEG/Chitosan combination after full spinal cord transection. No other intervention deployed over the past 30 years in similar SCI models reached the levels of recovery seen here.[18] We believe that this represents a revolutionary advancement in the treatment of SCI.

### Ethical approval

The study protocol was approved by the Local Ethics Committee of the Stavropol State Medical University (No. 97, April 15, 2021). This trial was carried out in accordance with the ethical standards of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The author(s) declare that they have taken the ethical approval from IRB.

### Declaration of patient consent

Patient's consent not required as there are no patients in this study.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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## COMMENTARY

In 1974, it was discovered how PEG evinced fusogenic properties on cell membranes. In 1981, Bittner first reported how single axons submitted to sharp transection could be restored ("refused") by applying PEG and in 1999, Borgens's group first reported on the capacity of PEG to refuse the axonal component of a severed spinal cord in guinea pigs.[19] Unfortunately, no one caught on to the potential of this technology for the repair of spinal cord injury (SCI) in man. Truth be told, for PEG to be effective, this has to be applied immediately after injury, which is a tall order in most circumstances. Besides, it was not clear what protocol might be leveraged in which PEG could be deployed. Furthermore, PEG comes in several molecular weights and dilutions, and these can affect the outcome.

This changed in 2013 with the introduction of the GEMINI spinal cord fusion protocol, a combination of topically applied fusogens (e.g., PEG) to transected cords combined with spinal cord stimulation – SCS.[7] Immediately after that, Canavero *et al*.[4,6] suggested that such a protocol might be applied to a previous approach developed by Dr Freeman in the 1960s[18], which required vertebral shortening and extirpation of the damaged level of the cord. Subsequently, it became clear how the same approach opened the way to spinal cord transplantation.[8]

Unfortunately, the SCI community as a whole spurned this approach (perhaps the prospect of having their funds rescinded bears some relevance here!), and basically, only the HEAVEN team and a few others pursued this approach in further animal studies with the goal of treating SCI. Recently, a group confirmed the efficacy of combined PEG and SCS in rodents.[23,24] Furthermore, PEG has been combined with other substances, e.g. ibuprofen, an anti-inflammatory drug, with the immediate recovery of motor-evoked potentials[22] or PLGA.[13]

Chitosan, as reported by Lebenstein-Gumovski et al, is another remarkable fusogen. A combination of PEG and Chitosan makes sense, and in fact, studies of PEG and photo-cross-linkable chitosan have been published in the past.[1,15,21] However, no one had studied the effects on a large animal model of spinal cord transection, as the Russian team now reports.

What needs discussion is the overall approach taken by the authors. PEG was also injected IV to maximize effects. Actually, studies of IV PEG in SCI dogs had mixed results [12,16], and in any case, we do not know what further benefit IV PEG might confers to local application alone.[7]

These authors applied topical hypothermia to the damaged cord with the aim of neuroprotection. Spinal cord hypothermia was first applied decades ago and is still being assessed clinically.[2] However, there is contradicting evidence on how this might affect PEG fusogenic potential, both in a detrimental or supportive direction.[11,14] However, since all these studies differ among them in many ways, it is not clear what conclusion to reach at this point regarding a human clinical trial. Certainly, further studies comparing groups of animals treated with local cooling or not are needed before clinical deployment.

The GEMINI protocol includes electrical stimulation (SCS, invasive or non-invasive, plus transcranial magnetic stimulation). In the present study, myostimulation was administered, which makes sense. Rehabilitation was also instituted. These authors should be commended for the combined approach.

How do locally injected fusogens work? We now know that the fusion of axons in the white matter only plays a secondary role, as motor fibers are not neatly distributed in bundles but, in real life, tend to be more widespread. For PEG fusion to work, one needs the exact same axon to be aligned without too much pressure for several minutes to achieve fusion, and one sees how this is clearly impossible to achieve. Instead, spinal cord fusion exploits one of GEMINI's principles, that is, the neuroprotectant effect on the gray matter (motor highway[2,5]) that allows immediate resprouting across the sectional area from sensory- and motor-coded gray matter interneurons, as shown in past studies[10,17] and confirmed in the current one. Instead, sensory-wise, the posterior columns are comparable to peripheral nerves, being bundles of axons only, and here, we know that fusion works – although haphazardly – to restore sensory transmission in minutes.[7]

We do not know whether the combination of PEG and chitosan is superior to either one, or these calls for ad hoc studies: teasing out the effects of each and possible synergistic effects is paramount. Parenthetically, we notice that Bittner's group recently reported that their fusion protocol is not superior to PEG alone.[9]

Another important point is that central pain was not reported by the authors, which is in line with all previous studies,[3,7] a key translational point.

Finally, PEG's potential to induce allergic reactions, including anaphylaxis, is associated with very high molecular weights, not PEG600, as used by these and other authors.[20]

In conclusion, I commend this Russian team for a meticulously conducted study in a large animal SCI model of a fusogenic combination. This small trial was conducted with a human trial in sight and is all the more informative given the highly relevant results. One can only wonder whether this study will finally convince SCI associations to sponsor a trial of this sort.

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