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Reviving matrix (anti-gliotic guiding regenerative gel) for reconstruction of severely injured peripheral nerve and spinal cord

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Abstract
Peripheral nerve injury (PNI) leads to partial or complete paralysis, severe pain, disabilities and deterioration in quality of life. PNI occurs in about 2.8% of all trauma patients. Incidence of nerve injuries is 13.9/100,000 inhabitants per year. The innovative Anti-gliotic Guiding Regenerative Gel (AGRG) is a special milieu that increases nerve growth and promotes recovery, aiming, ultimately, at restoring the function of an injured peripheral nerve and enable spinal cord repair. The AGRG is composed of: 1) anti-oxidant, found to protect cells from oxidative stress, regulating immune function, maintaining endothelial cell integrity; 2) proprietary peptide (16 amino acids) based on the active sites of laminin peptide and containing two penta-peptides, which acts as a scaffold for the nerve fibers to grow along; 3) hyaluronic acid, which is highly hydrated and contributes to the success of survival, growth and regeneration of nerve fibers by protecting them from drying; and 4) copaxone is an immunomodulatory and neuroprotective drug. These results show that treatment with AGRG was active in enhancing spinal repair in both acute and chronic SCI models in rats; highlighting that AGRG's superiority in promoting neuronal regeneration. AGRG opens a new modality in treatment of injured nerves, enabling the introduction of composite implant, with decreased time and improved quality of recovery, for clinical applications.

Background

Peripheral nerve and spinal cord injuries are debilitating conditions to civilians suffering motor vehicle accidents, or home, work and sporting injuries or acts of violence, as well as injuries occurring during military service.

Peripheral nerve injury (PNI) leads to partial or complete paralysis, severe pain, disabilities and deterioration in quality of life. PNI occurs in about 2.8% of all trauma patients. Incidence of nerve injuries is 13.9/100,000 inhabitants per year. The peripheral nervous system has, to some extent, an ability to regenerate axons after damage, however, reconnecting nerves to allow long distance regeneration across an injury site remains to this day a major clinical challenge [1].

The gold standard treatment for nerve loss longer than 2 cm is an autologous nerve graft procedure, by which nerve tissue from elsewhere in the body is harvested and grafted into the gap providing physical bridge for axon regeneration. Nerve autograft procedure is complex and technically demanding to manage, and in case of a severe injury, the results are often far from optimal. Absorbable nerve guides have been a promising alternative for autologous nerve graft repair, decreasing surgical time and avoiding donor site morbidity,

including sensation loss and/or cosmetic defect [2]. However, available nerve guides in the market are not able to bridge more than 2 cm long nerve loss, which represent the majority of severe injuries in clinical practice. Therefore, the nerve repair market is thus still in need of a simple and reliable off the-shelf product that can bridge major nerve gap ($>$ 2 cm).

Annually, nearly 500,000 people worldwide suffer a *spinal cord injury* (SCI), which results in an immediate and permanent loss of neurological sensory and motor function below the level of injury. SCI is accompanied by a scarring process resulting from the proliferation of astrocytes in the injured area, leading to formation of an astro-glial scar barrier, which is one of the most important factors that prevent axonal growth and therefore spinal cord recovery. Currently, to the best of our knowledge, there is no defined satisfactory treatment for SCI [3].

Uniqueness of Technology

The innovative Anti-gliotic Guiding Regenerative Gel (AGRG) is a special milieu that increases nerve growth and promotes recovery, aiming, ultimately, at restoring the function of an injured peripheral nerve and enable spinal cord repair. The AGRG is composed of: 1) anti-oxidant, found to protect cells from oxidative stress, regulating immune function, maintaining endothelial cell integrity; 2) proprietary peptide (16 amino acids) based on the active sites of laminin peptide and containing two penta-peptides, which acts as a scaffold for the nerve fibers to grow along; 3) hyaluronic acid, which is highly hydrated and contributes to the success of survival, growth and regeneration of nerve fibers by protecting them from drying; and 4) copaxone is an immunomodulatory and neuroprotective drug.

AGRG's unique properties are: it is transparent, highly viscous gel, almost impermeable to liquids and gasses, flexible, elastic, malleable, and adaptable to various shapes and formats. The AGRG resembles the extracellular matrix, supports and enhances axonal regeneration across nerve gap and enables reconnecting massive peripheral nerve loss defect, when used as a tube milieu. The AGRG showed efficacy in its ability to overcome the scar barrier, promote and enhance axonal sprouting and regain axonal fibers through the lesion area in the injured spinal cord.

AGRG opens a new modality in treatment of injured nerves, enabling the introduction of composite implant, with decreased time and improved quality of recovery, for clinical applications.

State of the Art

Peripheral nerve reconstruction – in a proof of concept in vivo study on peripheral nerves with massive nerve loss we showed previously a massive growth of myelinated axons and continuation of axonal sprouting through the nerve guide filled with the guiding regenerative gel, to the distal part of the nerve in a 15 mm critical gap in rats, which is not possible when bridging with an empty collagen nerve guide. Furthermore, it was shown to enable nerve regeneration comparable with the "gold standard"treatment (nerve autograft) with no significant difference [4]. Moreover, the guiding regenerative gel was shown to exhibit regaining of function to the left paralyzed limb following massive nerve loss of 15 mm in rats 6 month form the injury; while the "gold standard"treatment only supported limited movement, and an empty nerve guide was unable to support any movement [5, 6, 7].

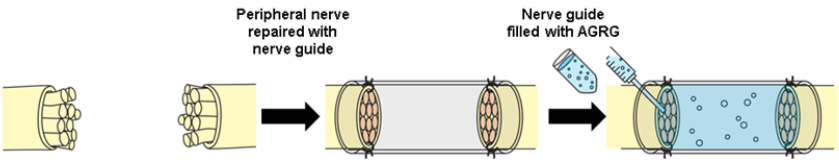

Fig. 1.: Model of Peripheral Nerve Injury and Reconstruction.

Recently, a chronic (delayed) PNI human related model in rabbits with a nerve defect of 25 mm critical gap, in which AGRG was applied (Fig. 1), showed a surprising electrophysiological beneficial effect of the AGRG when compared with the autologous nerve reconstruction.

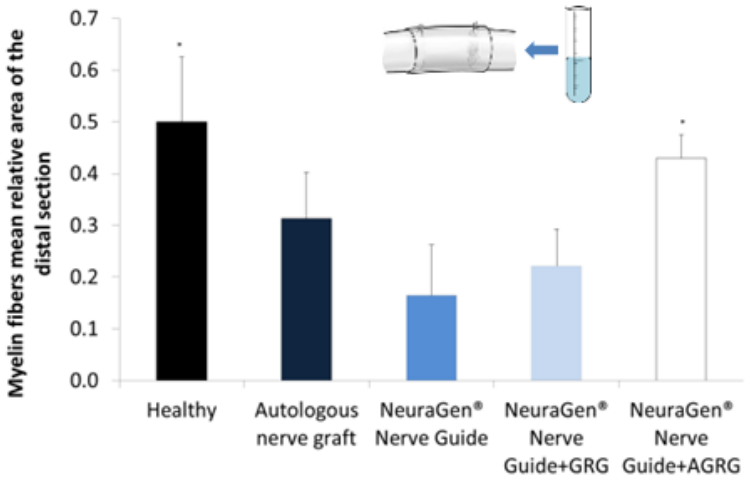

Fig. 2.: Histochemical evaluation of a chronic PNI in rabbits.

This trend was observed also thirty-one weeks post treatment surgery (delayed reconstruction) in the histochemical evaluation showing a significant regeneration when treated with AGRG, compared with the empty nerve guide and succeeded to significant regenerated the tibial portion of the sciatic nerve, as well as the autologous nerve graft and almost similar to healthy nerve [8] (Fig. 2).

The model used, rabbit model of the delayed (chronic) PNI with massive loss defect, represents the human condition (chronic, large gap).

The data shows that treatment with AGRG was active in enhancing peripheral nerve repair in a severe chronic PNI model in rabbits; highlighting that AGRG's superiority in promoting neuronal regeneration in current treatments.

Spinal cord reconstruction – in a recently conducted in vivo study evaluating the efficacy of AGRG in promoting nerve regeneration following complete SCI (removal of 2 mm spinal cord segment) in both acute and chronic rat models (Fig. 3).

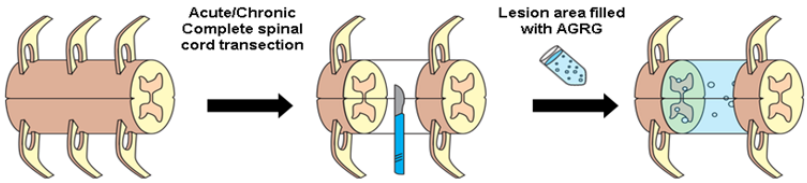

Fig. 3.: Model of spinal cord injury and reconstruction.

It was observed that AGRG was able to:

1. Improve movement in previously paralyzed limbs: after 21 weeks when treated with AGRG, the BBB score (Basso-Beattie-Bresnahan scale) in the acute SCI model reached to a score about 8 - the rat sweeping with no weight support or plantar placement of the paw with no weight support and some rats showed partial walking in AGRG group, while in the control group (untreated) the score reached to about 1.5.

   In the extreme SCI model (i.e. chronic) the BBB score 24 weeks after treatment was about 2 in the AGRG group; while in the control group the score was about 0.25.

2. Promote regaining of conductivity in the previously paralyzed limbs: regained conductivity (somatosensory evoked potentials) is evident in 75% of the paralyzed limbs in the AGRG group 24 weeks after acute SCI, while in the control group only 25% of the paralyzed limbs showed a signal.

3. Promote axonal penetration through the glial scar barrier: myelinated neuronal fibers were found in all cross sections of the injured spinal cord treated with AGRG in both SCI models.

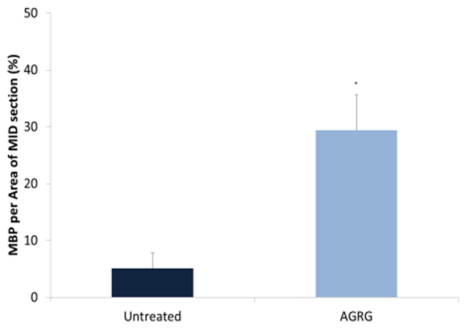

Fig. 4.: Acute SCI: Histochemical evaluation in rats (MBP – Myelin Basic Protein – stain)

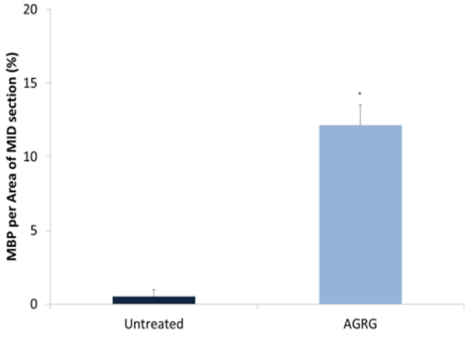

Fig. 5.: Chronic SCI: Histochemical evaluation in rats (MBP – Myelin Basic Protein – stain)

In the untreated group, minor sprouting of myelinated neuronal fibers was observed at the lesion area in the acute SCI model (Fig. 4) and almost no myelinated neuronal fibers were observed in the chronic SCI model (Fig. 5) [8].

Conclusion

These results show that treatment with AGRG was active in enhancing spinal repair in both acute and chronic SCI models in rats; highlighting that AGRG's superiority in promoting neuronal regeneration.

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