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New Aspects in Nerve Regeneration

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BACKGROUND

Peripheral nerve injuries represent a major cause of morbidity and disability worldwide. In addition to the economic burden on the national level, peripheral nerve injuries impose substantial costs on society in terms of long-term disability, reduced quality of life, and pain. It has been estimated that peripheral nerve injuries affect 2.8% of all trauma patients, many of whom acquire life-long disability. The annual incidence of peripheral nerve injuries in developed countries has been reported as 13 to 23 out of 100,000 persons. Recovery following severe peripheral nerve injury is often dismal, despite the inherent capability for axonal regeneration. Autologous nerve grafts are considered the gold standard treatment in cases of nerve defect, although often not providing satisfactory results. Moreover, autologous nerve donor may cause related neurological morbidity at the donor site, including possible neuroma formation. The use of nerve guidance channels (tubes), sutured in between the proximal and distal nerve stumps, has been actively pursued to obviate the need for the second procedure at the donor site and to obtain better regenerative results in comparison to the autologous nerve graft.

BIOARTIFICIAL GUIDES FOR PERIPHERAL NERVE REPAIR: DEVELOPMENTS AND FINDINGS FROM THE EUROPEAN COMMUNITY COLLABORATIVE PROJECT BIOHYBRID

The BIOHYBRID consortium consisted of Department of Clinical and Biological Sciences, Universita Degli Studi di Torino, Turin, Italy; Institute of Neuroanatomy, Hannover Medical School, Hannover, Germany; Center of Systems Neuroscience (ZSN), Hannover, Germany; Department of Translational Medicine and Hand Surgery, Skene University Hospital, Lund University, Malmц, Sweden; Division of Peripheral Nerve Reconstruction, Department of Neurosurgery, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel; Institute of Neurosciences and Department of Cell Biology, Physiology and Immunology, Universitat Autтnoma de Barcelona, and CIBERNED, Barcelona, Spain; Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; NVR Research Ltd, Ness-Ziona, Israel; and Medovent GmbH, Mainz, Germany, was build up with the overall aim to develop, in a preclinical perspective, an innovative biohybrid artificial nerve. The developed Chitosan nerve conduits (Reaxon® Nerve Guide) allow functional and structural regeneration across a 10-mm sciatic nerve gap in rats. In 57% of the animals also regeneration across 15-mm gaps was allowed.[1]

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COMPARISON OF RESULTS BETWEEN CHITOSAN HOLLOW TUBE AND AUTOLOGOUS NERVE GRAFT IN RECONSTRUCTION OF PERIPHERAL NERVE DEFECT

A chitosan tube for regeneration of the injured peripheral nerve in a rodent transected sciatic nerve model was evaluated in comparison to autologous nerve graft repair.[2] Chitosan hollow tube was used to bridge a 10 mm gap between the proximal and distal ends in 11 rats. In the control group, an end-to-end coaptation of 10 mm long autologous nerve graft was performed in 10 rats for nerve reconstruction. Sciatic functional index (SFI) showed an insignificant advantage to the autologous group both at 30 days ($p=0.177$) and at 90 days post procedure ($p=0.486$). Somato-sensory evoked potentials (SSEP) and compound muscle action potentials (CMAP) tests showed similar results between chitosan tube (group 1) and autologous (group 2) groups with no statistically significant differences. Both groups presented the same pattern of recovery, with 45% in group 1 and 44% in group 2 ($p=0.96$) showing SSEP activity at 30 days. At 90 days most rats showed SSEP activity (91% vs 80% respectively, $p=0.46$). The CMAP also demonstrated no statistically significant differences in latency (1.39ms in group 1 vs 1.63ms in group 2; $p=0.48$) and amplitude (6.28mv vs 6.43mv respectively; $p=0.8$). Ultrasonography demonstrated tissue growth inside the chitosan tube. Gastrocnemius muscle weight showed no statistically significant difference. Histomorphometry of the distal sciatic nerve, 90 days post reconstructive procedure, showed similar number of myelinated fibers and size parameters in both groups ($p\geq0.05$). In conclusions, chitosan hollow tube used for peripheral nerve reconstruction of rat sciatic nerve showed similar results in comparison to autologous nerve grafting.

TISSUE ENGINEERED GUIDING REGENERATIVE GEL FOR RECOVERY OF PERIPHERAL NERVE INJURY WITH MASSIVE LOSS DEFECT

Many severe peripheral nerve injuries can only be treated through surgical reconstructive procedures. Such procedures are challenging, since functional recovery is slow and can be unsatisfactory. One of most promising solutions already in clinical practice is synthetic nerve conduits connecting the ends of damaged nerve supporting nerve regeneration. However, this solution still does not enable recovery of massive nerve loss defect.

The present composition of Guided Regenerative Gel (GRG), offers opportunities for the development of a novel biocompatible gel for recovery of peripheral nerve with massive loss defect, enhancing axonal growth and nerve regeneration. It is composed of a complex of substances comprising transparent, highly viscous gel that is almost impermeable to liquids and gasses, flexible, elastic, malleable and adaptable to various shapes and formats. The gel resembles the extracellular matrix (ECM) and was found to support three dimensional growth and differentiation of various cell types.

Pre-clinical proof of concept in peripheral nerve damage rat model, conducted under supervision of Teva Pharmaceutical Industries, showed that GRG enhanced nerve regeneration when placed in nerve conduits enabling recovery of massive nerve loss, previously unbridgeable.

Potential applications of GRG include tissue reconstruction and cell therapy; Regeneration of massive regional losses in tissues and organs; Nerve healing, wound healing, and preservation of muscles and other human and animal tissues from deterioration; Combining efforts with anti-gliosis, anti-scaring and anti-barrier formation agents. The final goal is to bring this technology to the clinic. Hence, the GRG technology with the additional suggested improvements is a promising platform for stem cells therapy, spinal cord injury, stroke and Parkinson's disease.

MUSCLE RESPONSE TO COMPLETE PERIPHERAL NERVE INJURY: DYNAMIC CHANGES OF ACETYLCHOLINE RECEPTORS (ACHR) AND CREATINE KINASE (CK) ACTIVITY OVER TIME

Post-traumatic prevention of muscle atrophy is a major challenge in restorative medicine. When muscles are denervated,

as in cases of complete peripheral nerve injury, they deteriorate progressively. Denervated muscles can account for significant differences in the extent of AChR and CK activity during the denervation period.

A study by Rochkind et al., was designed to assess the status of skeletal muscles during long-term denervation processes, by investigating changes in the level of AChR and CK activity in the denervated gastrocnemius muscle of the rat.[4] The study was conducted on 48 rats. The gastrocnemius muscle was denervated by removing a 10mm segment of the sciatic nerve. Under general anesthesia, the rats were euthanized at seven time points: 7, 14, and 21 days, and 1, 2, 4, and 7 months. AChR was quantified by the 125I-a-bungarotoxin. CK activity was measured by a specific spectrophotometric method.

Muscle denervation results is progressive degradation of AChR and CK content. After 4 months for AChR and 2 months for CK content we found partial preservation in both components until 7 months after complete muscle denervation. Our study showed that survival of denervated muscle is longer than previously considered. Partial preservation of AChR and CK activity is seen in long-term denervated muscle. Therefore, maintaining CK activity and the amount of AChR suggests a possibility for muscle reinnervation following nerve reconstruction procedure.

EFFECTIVENESS OF LOW LEVEL LASER THERAPY FOR PERIPHERAL NERVE RECOVERY AND RELATED MOTOR FUNCTION

Considerable interest exists in the potential therapeutic value of laser phototherapy for restoring or temporary preventing denervated muscle atrophy as well as enhancing regeneration of severely injured peripheral nerve.

Laser phototherapy was applied for treatment of rat denervated muscle, as well as on rat sciatic nerve model after crush injury, direct or side-to-end anastomosis and neurotube reconstruction.[5] Nerve cells' growth and axonal sprouting were investigated on embryonic rat brain cultures. The animal outcome allowed clinical study on patients suffering from incomplete peripheral nerve injuries.

In denervated muscle, animal study suggests that function of denervated muscles can be partially preserved by temporary prevention of denervation–induced biochemical changes. The function of denervated muscles can be restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury.

In peripheral nerve injury, laser phototherapy has a protective and immediate effect, it maintains functional activity of the injured nerve, decreases scar tissue formation at the injury site, decreases degeneration in corresponding motor neurons of the spinal cord and significantly increases axonal growth and myelinization.

In cell cultures, laser irradiation accelerates migration, nerve cell growth and fiber sprouting.

In a pilot, clinical, double-blind, placebo-controlled randomized study in patients with incomplete long-term peripheral nerve injury, 780-nm laser irradiation can progressively improve peripheral nerve function, which leads to significant functional recovery.

In conclusion, based on the animal and clinical studies which showed the promoting action of laser therapy on peripheral nerve regeneration, it is possible to suggest that the time for broader clinical trials has come. Laser activation of nerve cells, their growth and axonal sprouting can be considered as potential treatment of neuronal injury.

In Conclusion:

Repair and regeneration of damaged peripheral nerves is a major field where innovative therapies in regenerative medicine can be applied at the clinical level. Recent development in the field of artificial conduits and extracellular matrix, enabled to create an innovation artificial nerve, where application of laser phototherapy can stimulate axonal growth into the nerve. This developments are promising in their ability to benefit the many patient suffering from disabilities related to sustained peripheral nerve injuries.

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Шимон Роххінд

Нові аспекти щодо регенерації нервів

Проф. Шимон Роххінд, дипломований лікар, доктор медичних наук, Директор відділу периферичного нервового відновлення, відділення нейрохірургії, Директор науково-дослідницького інституту по реконструкції нерва

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Резюме. Пошкодження периферичних нервів представляють собою основну причину захворюваності та інвалідності в усьому світі. На додаток до економічних проблем на національному рівні, пошкодження периферичних нервів накладають суттєві витрати на суспільство з точки зору довгострокової інвалідності, зниження якості життя та болю. Було підраховано, що ушкодження периферичних нервів вплинули на 2,8% усіх травмованих пацієнтів, більшість з яких отримують довічну інвалідність. Щорічні випадки ушкоджень периферичних нервів у розвинених країнах, згідно з повідомленнями, складають від 13 до 23 на 100000 чоловік. Відновлення після важкої травми периферичних нервів є часто катастрофічним, не зважаючи на вроджені можливості для регенерації аксонів. Аутологічні нервові трансплантати вважаються золотим стандартом лікування у випадках дефекту нерва, хоча часто не забезпечують задовільних результатів. Більш того, донорство аутологічного нерва може викликати споріднені неврологічні хвороби зі сторони донора, у тому числі можливого утворення невроми. Використання провідних каналів (труб) нерва, зшитих у проміжку між ближніми і далекими куксами нервів, активно продовжувалось для уникнення необхідності другої процедури для донора, а також для отримання кращих регенеративних результатів у порівнянні із аутологічним трансплантатом нерва.

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