main source of MSCs for both experimental and clinical applications. However the number of bone marrow MSCs significantly decreases with age and the bone marrow HLA compatible donors are very difficult to find, which makes the search for adequate alternative sources of these cells necessary for autologous and allogenic use. The stem cells obtained from the blood and Wharton´s jelly of the umbilical cord are a promising source of stem cells: i) the number of stem cells per volume is higher than in bone marrow, ii) a complete or high HLA profile match for allogenic use is not necessary, which permits to greatly enlarge the number of available donors iii) the stem cells are easier to obtain, manipulate and criopreserve, and their collection in ethically approved by national and international laws.

A multidisciplinary team, including Veterinaries, Engineers, Medical doctors like neurologists and surgeons through Experimental Surgery has a crucial role in the development of biomaterials associated to these cellular systems, and in testing the surgical techniques that involve their application, always considering animal welfare and the most appropriate animal model. Several biomaterials developed by our research group (including PLGA with a novel proportion 90:10 of the two polymers, poly(L-lactide):poly(glycolide), hybrid chitosan and collagen) have been tested associated to cellular systems to promote nerve regeneration after axonotmesis and neurotmesis injuries in the sciatic nerve experimental model. The cellular systems that have been studied in this context include an immortalized neural cell line N1E-115, stem cells obtained from the Wharton´s jelly of the umbilical cord, CD34+ stem cells from the umbilical cord blood, and MSCs from umbilical cord matrix. The tube-guides associated to one of the cellular systems are tested in the rat sciatic nerve across a 10 mm-gap (neurotmesis) or in a 3 mm axonotmesis lesion. The cells introduced are able to produce growth factors in the local of the nerve injury, during the necessary healing period.

Under general anesthesia, the sciatic nerve is unilaterally exposed. After nerve mobilization, a transection injury is performed, just above the terminal nerve ramification (neurotmesis). For the crush injury, a non-serrated clamp exerting a force of 54N is used for a period of 30 seconds to create a 3 mm long crush injury, 10 mm above the bifurcation. For reconstruction, the biodegradable tubes/membranes covered by the cellular system are used, for a nerve gap of 10 mm or to involve the axonotmesis lesion area, respectively. Motor functional recovery after the sciatic nerve reconstruction is assessed serially using video recording of the gait for biomechanical analysis, by measuring extensor postural thrust (EPT), sciatic functional index (SFI) and sciatic functional index under static conditions (SSI). The sensitive recovery is tested by the withdrawal reflex latency (WRL) and von Frey filaments. The repaired nerves are processed for light and electronic microscope analysis, imunohistochemistry, confocal microscopy and stereological studies. Functional and morphologic results obtained with these different biomaterials and cell lines are presented and discussed in terms of better improvement of nerve regeneration after axonotmesis and neurotmesis injuries in rat sciatic model.

Biodegradable hydrogels as scaffolds for nerve regeneration

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Transected peripheral nerves are typically reconnected by direct end-to-end surgery or by autologous nerve graft. However, artificial synthetic guide are a successful alternative which may prevent neuroma formation (1). Among biodegradable conduits a novel approach is represented by use of tuneable polyamidoamine (PAA)-based hydrogels, with specific diameters, different shapes and/or dimensions. Depending by their crosslinking degree, hydrogels made by PAAs are tough material which may absorb large amounts of water. PAA hydrogels are biocompatible and biodegradable in vitro to non-toxic low molecular weight products over a period of time varying from few weeks to months (2). In order to evaluate their ability to promote nerve regeneration, PAA hydrogels scaled as scaffold conduits (10mm lenght, 1mm internal diameter) were studied by using an experimental model of rat nerve transection. A conduit was used to join a gap of 4-5 mm in the sciatic nerve, and a longitudinal analysis was made at 30, 45, 60, 90 days post-surgery. We performed the gait analysis to evaluate locomotor coordination, the plantar test to study nociception and pain sensitivity, and the morphological-morphometric analysis to evaluate the nerve recovery. Preliminary results indicate that nerve ends can be successfully joined by these PAA-based hydrogel conduits.

One month after surgery, in fact, the regeneration is appreciable inside the conduit and the nerve is resistant to mechanical traction, without signs of inflammation or serum infiltrate. In the implanted rats 45 days after surgery the footprints analysis reveals a trail similar to sham-operated animals, while the thermal hypersensitivity tend to normalize to the control levels at later times. The

morphological evaluation of the explanted conduit at 90 days after surgery shows normal myelin structures, confirming nerve regeneration and complete scaffold re-absorption. In conclusion, our results demonstrate that PAA hydrogels might be a promising scaffold tube for nerve regeneration. Further studies on the hydrogels functionalization for drug delivery, with growth factors or hormones, are in progress in our labs.

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Tissue engineered guided regenerative gel for recovery of peripheral nerve injury with massive loss defect

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Background

Guided Regeneration Gel (GRG) was developed to simulate the extracellular milieu, support growth and activity of axons and cells in vitro and in vivo upon implantation, as well as destined to serve as a regenerative and repair source for nerve tissue reconstruction.

Purpose

Evaluation of the efficacy of GRG based on tissue-engineering technology for the treatment of complete peripheral nerve injury with significant loss defect.

Methods

Rat sciatic nerve was completely transected and a 2 cm segment of the peripheral nerve was removed. Composite transplant, containing a guiding tube filled with GRG, which is an excellent milieu for growth of axons, was placed between the proximal and the distal parts of the transected nerve for reconnection of 2cm long distance.

Results

The post-operative follow-up (up to 4 months) of the operated rats showed re-establishment of active foot movements. The tube had dissolved and the nerve showed complete reconnection. Histological observation of the nerve showed growth of myelinated axons in the place where nerve defect was replaced by composite nerve transplant, and continuation of axonal sprouting through the place of the tube to the distal part of the nerve.

Conclusion

Utilization of an innovative composite implant to bridge a gap resulting from removal of a 2cm peripheral nerve segment shows promise, suggesting the feasibility of this approach for reconstruction of peripheral nerve lesions. Such an implant may serve as a vital bridging station in peripheral nerve injuries with massive loss of tissue.

Ciliary neurotrophic factor induces more extensive collateral sprouting of motor than of afferent axons associated with improved functional reinnervation of the biceps muscle in an experimental model of end-to-side neurorrhaphy

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Introduction

End-to-side neurorrhaphy is based on collateral sprouting of an intact axon. The aim of the present study was to quantitatively assess collateral sprouts sent out by intact motor and sensory axons. End-to-side neurorrhaphy of the distal stump of transected musculocutaneous nerve (MCN) with intact ulnar nerve (UN) was performed in a rat model.

Material and Methods

The experimental model was used to evaluate the efficacy of Cerebrolysin and ciliary neurotrophic factor (CNTF) treatment in promoting the reinnervation of MCN stump by collateral sprouts of intact afferent and motor axons of the UN. CNTF, Cerebrolysin and PBS (control) were