

**ACETYLCHOLINE RECEPTORS OF THE NEUROMUSCULAR JUNCTIONS
PRESENT NORMAL DISTRIBUTION AFTER PERIPHERAL NERVE INJURY AND
REPAIR THROUGH NERVE GUIDANCE ASSOCIATED WITH HETEROLOGOUS
FIBRIN BIOPOLYMER.**

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Peripheral nerve injuries (PNIs) cause changes beyond the area of injury (nerve) and the effector organ (skeletal muscle), it can also affect the neuromuscular junctions (NMJs) leading to the disaggregation of acetylcholine receptors (AChRs), which might occur due to alterations in the agrin-LRP4 pathway. One of the techniques used for the repair of this type of injury is nerve guidance, where a conduit serves as a guide for the reconnection of nerve stumps. In order to potentiate the effect of this technique, co-adjuvant substances can be added, such as heterologous fibrin biopolymer (HFB) CEVAP (UNESP/BRASIL) which has a hemostatic capacity and can be used as a scaffold. The objective of this study was to evaluate the effect of the association of nerve guidance with HFB after PNIs on AChRs and associated proteins. Forty male Wistar rats (CEUA/FMB 1244/2017) were divided into 4 groups: CS (control-sham: right ischiatic nerve exposure), CD (control-developed: 8mm gap neurotmesis), PCL (neurotmesis and fixation of stumps in polycaprolactone-PCL-12mm tube) and HFB (neurotmesis and fixation of stumps in polycaprolactone-PCL-12mm tube associated with 100µl of BHF). Ninety days after the surgical procedure the animals were euthanized, and the right soleus muscles were removed. The S100 and LRP4 proteins were quantified via Western Blotting, and AChRs were immunostained by rhodamine-conjugated alpha-bungarotoxin and photographed using Laser Scanning Confocal Microscope (Leica). The images were morphometrically analysed via Image J using the NMJ-morph platform, which allows a standardized and reliable analysis of NMJs. We found that the HFB showed similar NMJ morphology compared to CS group in terms of AChR area, motor plate area, and compactness. The same trend was observed in the results found for the AChRs area. However, the PCL group differed statistically from the CS. The S100 and LRP4 protein levels were increased in the HFB group compared to CS, while the PCL group presented intermediate values between the CS and HFB groups. The morphometric data found for NMJ showed that the HFB group approached the Control group with a normal distribution of the AChRs. This can be justified by the high values of S100 and LRP4 which constitute one of the nerve regeneration pathways. It is possible to conclude that the association of HFB with PCL can contribute to regeneration. Considering that LRP4 has the function of activating a cascade for neuromuscular synapses regeneration [1], where Schwann cells coordinate the realignment of axons in regeneration.

K. D. Gribble; L. J. Walker; , L. Saint-Amant; J. Y. Kuwada and M. Granato, "The synaptic receptor Lrp4 promotes peripheral nerve regeneration", *Nature communications*, **9**(1), 1-12 (2018).