

A Comprehensive Protocol for the Study of Sciatic Nerve Regeneration in the Rabbit

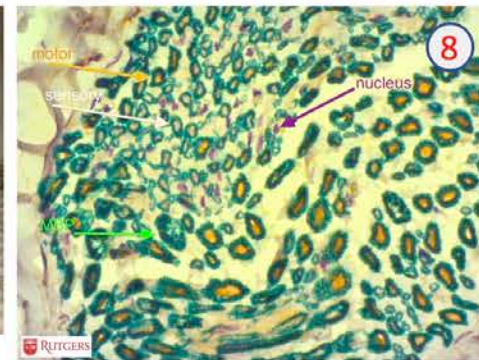
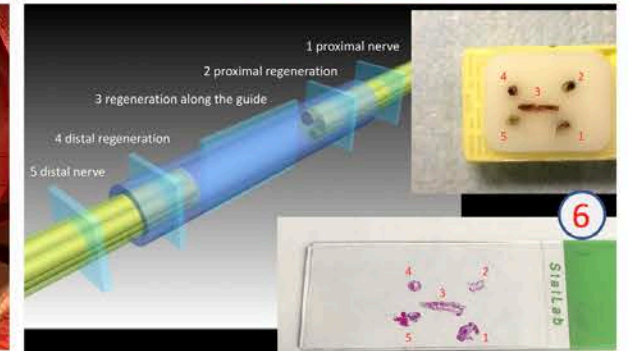
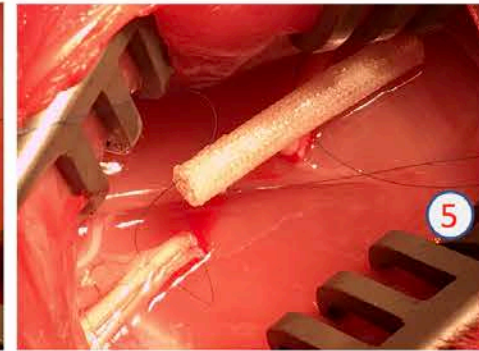
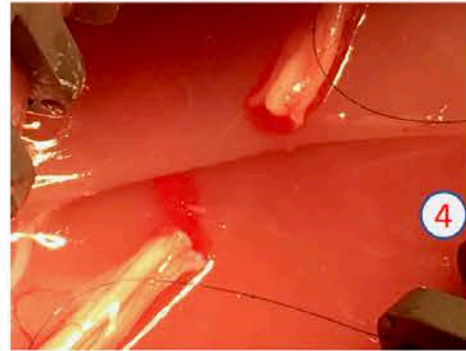
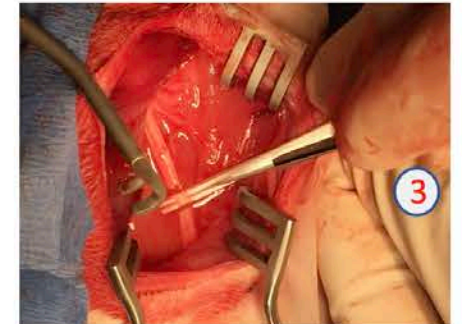
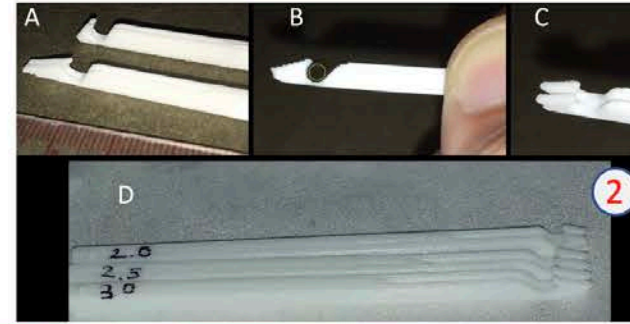
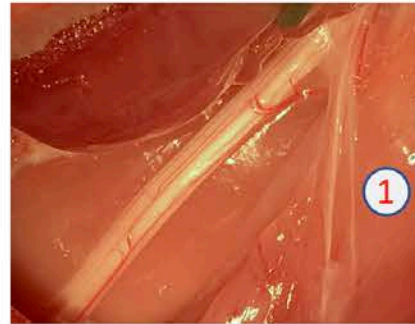
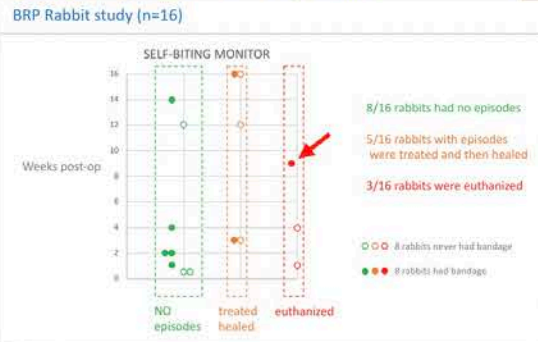
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Introduction. The majority of in-vivo studies on nerve regeneration have been performed in the Rat sciatic model; it is relatively cheap, so many animals can be associated to each experimental-point for a better statistical significance. However, there are limitations with the Rat sciatic model because its small dimensions restricts the range of length and diameter of the artificial guides to be tested. The use of bigger animals, like sheep or pig or non-human primate, is significantly more expensive; so, these more translational models are used at a second experimental stage which follows the experimentation in the rat.

Materials and Methods. We defined what is needed for an effective gap-injury model to test artificial nerve-guides in rabbit sciatic nerve gap-injury. We aimed at describing a comprehensive model which may provide: 1-a reduced number of steps in production and repair of the lesion; we used a double-arm suture for a faster anchoring and accommodation of the nerve stumps inside the guide. 2-an easily reproducible technique to produce a sharp transverse cut in sectioning the nerve; 3-a standard retrieval and histological procedure where both Myelin Basic Protein (MBP) and choline acetyltransferase (ChAT) were stained together with sufficient contrast on paraffin-embedded sections (reciprocal nerve staining RNS). 3D printed tools assisted both surgery and retrieval process. All our concepts and designs were tested in 16 rabbits (plus 2 refinement surgery).

Results. We were attracted by the potential translational capability of the New Zealand White Rabbit sciatic nerve model. It allowed to use diameters and lengths which are close to the standard practice in human Hand and Wrist. Costs for the rabbit model were higher than the rat but still quite reasonable; about \$150 for a Wister Rat and \$300 for a New Zealand White rabbit. Operation time (from incision to suture) was around 35 (+/-8) minutes (including time for imaging documentation). Throughout the procedure the animal remained in good conditions. The recovery occurred in less than 24 hours (animal feeding, drinking and re-establishing gait). Episodes of self-biting were observed in about half of the cases but they were controlled by a mix of prevention (by collar/bandaging) and antibiotic therapy (graph); one animal missed the time-point (arrow) and was rescheduled. Histology: sections were at proximal and distal stumps; proximal and distal regenerate; longitudinal central regenerate.

Conclusions. The Rabbit sciatic model is more demanding than the Rat sciatic model in terms of regulatory issues (Rabbit is a USDA species). However, it is more translational as far as dimensions of the implants are considered for an application in the hand and wrist (and other small-nerve sites). With our protocol, the Rabbit sciatic model can be proposed as a new standard procedure in the study of nerve regeneration assisted by artificial devices.



- 1-the Rabbit sciatic nerve and its epineural vascularization.
- 2-the 3D printed tool for the sharp cut (see ASPN 2018 meeting).
- 3-retraction of the nerve stumps soon after the sharp cut.
- 4-the circular cross-section of the main fascicles of the sciatic nerve as appears after the sharp cut.
- 5-a double-arm 7-0 monofilament nylon suture anchors the braided artificial nerve-guide to the epineurium.
- 6-the five region of interest in the histological analysis of the nerve regeneration and their standard assembly into the embedding block and, then, on the slide.
- 7-an e-collar post-operation and a bandaging of the operated limb were useful in prevention and treatment of self-biting.
- 8-the Reciprocal Nerve Staining (RNS) developed to identify Myelin Basic Protein (MBP) and choline acetyltransferase (ChAT) together and with sufficient contrast on paraffin-embedded sections (J Neurosci Methods. 2019 Jan 1;311:235-238)