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A simple and reliable method to perform biomechanical evaluation of postoperative nerve adhesions

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HIGHLIGHTS

- We investigated two methods to induce perineural scar tissue in animal models: epineural injury and muscular bed injury.
- We have developed a simple and reliable method for biomechanical evaluation of perineural scar tissue formation.
- Both methods for inducing perineural scar tissue formation were effective in providing a reliable experimental model for the study of anti-adhesion strategies.
- Multimodal analysis, by means of both qualitative and quantitative assessment, is needed for the study of post-surgical scar tissue formation in peripheral nerves.

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ABSTRACT

Background: Perineural fibrotic adhesions are among the major complications of peripheral nerve surgery. While different experimental models have been used for the pre-clinical testing of anti-adherential strategies, the methods used so far to induce scar tissue appear to be poorly standardized and reproducible.
New method: Thirty adult mice were used. Two methods were tested: the first one is based on burning the perineural muscular bed with a diathermocoagulator, while the second is based on direct scratching of the nerve surface with a cotton swab. After 3 weeks, the fibrotic reaction was assessed by measuring the peak pull out force of the nerve from muscular bed by means of a new tool specifically devised for biomechanical assessment of scar tissue formation. Moreover, histological analysis with specific collagen stain was also carried out.
Results: Both methods produced fibrotic reaction. Statistical analysis of biomechanical data showed a significant difference between burning and scratching group compared to the control sham operated group. No significant differences were detected between burning and scratching group. Histological analysis showed the presence of perineural scar tissue in both groups, though with a different distribution pattern.
Comparison with other methods: This protocol is easier to perform. The tool used for biomechanical evaluation is reliable and cheap.
Conclusions: Both methods for perineural scar formation are effective and simple. They represent reproducible models for the study of the anti-adherential strategies. Yet, biomechanical testing with the device that we have developed proved to be a reliable and simple method for the quantitative assessment of the degree of perineural adhesion formation.

1. Introduction

Postsurgical perineural scar is one of the most frequent causes of compressive nerve syndromes (Jones et al., 2012). To prevent this pathological condition, various treatment strategies have been proposed as barriers against perineural adhesions that can be applied

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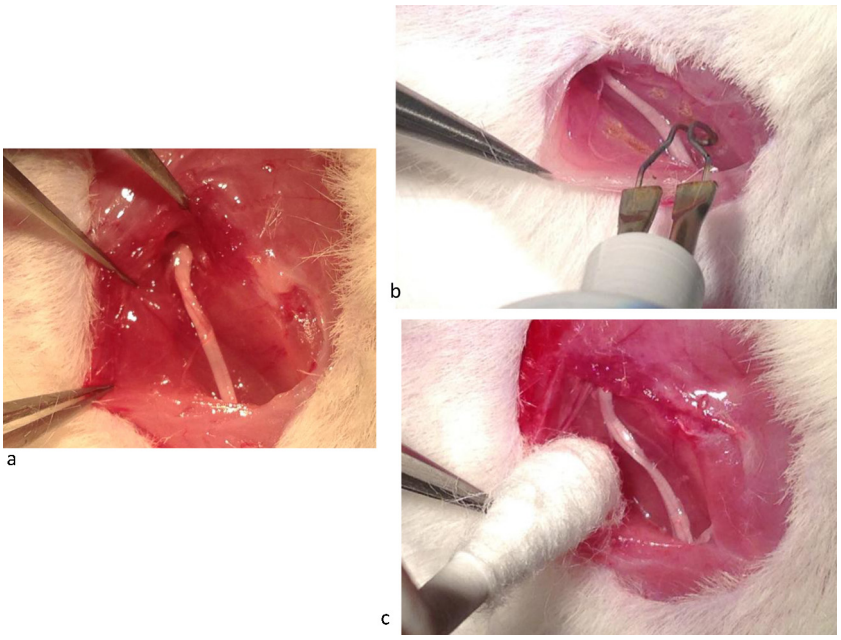

Fig. 1. Microscopical view of the gluteal splitting approach (a). Burn injury of muscle bed with diathermocoagulator (b). Scratch injury of sciatic nerve using cotton swab.

after nerve surgery (Smit et al., 2004; Dam-Hieu et al., 2005; Abe et al., 2005; Yamamoto et al., 2009). Whereas various techniques and protocols have been described to induce a perineural scar in experimental models for the pre-clinical testing of anti-adherence procedures, a recognized standardized protocol has not been defined yet (Smit et al., 2004). This makes it difficult to compare the efficacy of the different anti-adherence strategies in order to optimize clinical treatment.

Two frequently used methods to induce scar formation can be found in the literature: the first one consists in a direct lesion (mechanical, thermal or chemical) applied to the surface of the nerve (Smit et al., 2004; Dam-Hieu et al., 2005; Zuijdendorp et al., 2008); the second one consists in inducing a lesion of surrounding muscular bed which indirectly causes a damage to the nerve surface (Ikeda et al., 2003; Abe et al., 2005; Yamamoto et al., 2009).

Moreover, two different methods have been commonly used for the evaluation of perineural scar: morphological analysis, either macro and/or microscopic (Park et al., 2011; Dam-Hieu et al., 2005; Abe et al., 2005; Yamamoto et al., 2009) and functional biomechanical analysis (Ikeda et al., 2003; Smit et al., 2004; Abe et al., 2005; Zuijdendorp et al., 2008; Yamamoto et al., 2009). Finally, also regarding animal models, there is no homogeneity in the literature since different species have been used so far (Ikeda et al., 2003; Abe et al., 2005; Zuijdendorp et al., 2008; Yamamoto et al., 2009).

In order to identify a shared model for postsurgical perineural scar investigation, we aimed to develop a reproducible and standardized protocol in the mouse sciatic nerve model. To this end, we have developed a simple tool for biomechanical analysis that we associated to histological assessment in order to obtain both quantitative and qualitative description of scar distribution pattern.

2. Materials and methods

All procedures performed were in accordance with the Local Ethical Committee and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Thirty male mice (5 weeks old, average weight 28 g, Charles River Laboratories, Lecco, Italy) were used in this study. After intra-peritoneal anesthesia with ketamine 100 mg/kg + Xylazine 15 mg/kg, under microscopic magnification, both sciatic nerves were exposed by gluteal splitting incision to view clearly the sciatic nerve from the gluteal vein to trifurcation (Fig. 1a). Then, each nerve was randomly assigned to one of the three experimental groups: burning group (1), scratching group (2), control group (3).

Burning group: After retraction of the nerve the muscle surface was burnt with diatermocoagulator for about 0.8 cm along the nerve bed (Fig. 1b), as previously described (Ikeda et al., 2003; Abe et al., 2005; Yamamoto et al., 2009).

Scratching group: With a cotton brush, 20 bites were made on the external surface of the nerve for about 0.8 cm (Smit et al., 2004; Dam-Hieu et al., 2005; Zuijdendorp et al., 2008). The technique is illustrated in Fig. 1c.

Control group: The nerve was exposed and the skin was closed immediately after.

Animals were housed with standard light conditions and unlimited access to food and water. After 3 weeks all animals were sacrificed by cervical dislocation. In each group, biomechanical evaluation was performed. Three nerves for each group were not tested biomechanically in order to be processed for histological evaluation.

Biomechanical evaluation was performed to measure the peak pull out force of the nerve from the muscular bed. An original instrument adapted from previously ones was developed (Fig. 2a) (Ikeda et al., 2003; Smit et al., 2004; Abe et al., 2005; Zuijdendorp et al., 2008; Yamamoto et al., 2009). The sciatic nerve was exposed near to the origin and loaded with 9-0 Nylon suture at the beginning of scar tissue (Fig. 2b). Then, the suture was connected to a plastic can by a simple knot. The proximal end of the sciatic nerve was cut to 9-0 suture. Afterwards, the distal end of the sciatic nerve was exposed and cut.

The traction on the nerve increased gradually by means of a constant water flow filling up the plastic can. The water flow was kept constant at 100 ml/min and it was stopped when the nerve was definitely detached from his muscular bed. The peak pull out force was measured as the total weight of plastic can filled by water.

For histological analysis, the posterior space of the tight with nerve and scar tissue inside the muscles was harvested en bloc. The proximal end was marked with 9-0 Nylon. After paraffin inclusion (Raimondo et al., 2009), transversal sections (11 μm thickness)