



Short Communication

Use of Rubber Tree (*Hevea brasiliensis*) Latex Biomembrane in Diaphragmatic Injuries in Rabbits – an Experimental Study

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Submitted July 12th 2010, Accepted September 8th 2010

Abstract

The aim of this study was to evaluate the surgical use of the natural latex biomembrane in diaphragmatic injuries produced experimentally in rabbits. Fifteen healthy adult male and female New Zealand rabbits were employed. The rabbits were assigned to the experimental groups I, II, III, IV and V and analyzed on the 15th, 30th, 45th, 60th and 90th days post surgery, respectively. The surgical procedure consisted in the access to the diaphragm at the eighth right intercostal space, removal of a circle portion of approximately 1.5 cm in diameter following surgical repair with a latex membrane. Macroscopically, it was observed an excellent healing process during the experimental period. The clinical observations, complemented by the histological analysis, indicate that the latex membrane is useful for repair of traumatic injuries of the diaphragm of rabbits.

Key words: Biomembrane, cicatrization, diaphragm, implant, latex, rabbit.

The incidence of diaphragmatic hernias is elevated in the veterinary hospital routine of small animals (5). The main cause of injuries in the diaphragm muscle is trauma, as consequence of car accidents (1, 10).

Biological tissue is employed for reconstruction of diaphragm as in other organs. On the other hand, synthetic or natural materials have been used, including the latex. The latex biomembrane, for a therapeutic use, is made with the latex extracted from the rubber tree (*Hevea brasiliensis*). The membrane can be bathed with polylysine, a polycation that increases the micro-vascular permeability and blood flow. The treated biomembrane presents important biological properties such as: neoangiogenic activity, induction of cellular adhesion and formation of cellular matrix (6), acceleration of the repair, providing a decrease in the treatment time with a substantial economical advantage; it also offers the advantage of a reduced risk of disease

transmission in relation to the materials originated from animal tissues (7).

This study was undertaken to evaluate the surgical use of latex biomembrane in diaphragmatic injuries produced experimentally in rabbits.

The study was approved by the Bioethics Committee of the Universidade do Estado de São Paulo - Unesp, Jaboticabal - under the number 025416-07 and Universidade de Marília - Unimar.

Fifteen adult New Zealand rabbits - ten male and five female, with weights ranging from 3.0 to 3.5 kg, were employed. Five groups of three animals each were formed. All animals underwent a surgical procedure of diaphragmatic lesion, accessed through the eighth right intercostal space. The lesion consisted of the removal of a disk-shaped flap, from the muscular part of the diaphragm (about 1.5 cm diameter), following repair carried out with a latex membrane donated by Professor Joaquim Coutinho Netto, Department of Biochemistry and Immunology, FMRP-

USP. This membrane is produced and marketed by Pele Nova Biotecnologia S/A. The suture between the biomembrane and the diaphragm was performed in a simple continuous way with polyglactin 910. The external surgical wound underwent daily local cleaning with gauze and saline solution followed by compressive bandage. After seven days, the skin stitches were removed.

The euthanasia was carried out by the administration of Thiopental at a dose of 30 mg/kg (IV) followed by potassium chloride at a dose of 100 mg/kg (IV). The animals from group I were euthanized in the 15th day; group II in the 30th day; group III in the 45th day; group IV in the 60th day and the animals from group V in the 90th day.

After euthanasia, fragments of diaphragm were collected from the implant site, fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Sections of 5 µm thickness were cut for hematoxylin/eosin and Masson's trichrome for histopathologic evaluation.

The access to the diaphragmatic muscle by the eighth intercostal space was appropriate, allowing the fixation and suturing of the graft to the diaphragm muscle and excellent visualization of the surgical field (4).

The polyglactin 910 thread was shown to be sturdy and safe for fixing the membrane/muscle (5, 10). The simple continuous stitch showed easiness, rapid application and appropriate post-operative diaphragmatic defect sealing. It was verified the absence of suture dehiscence, without apparent impairment of the local blood supply (4, 10).

The tissue formation verified in the animals euthanized at the 15th day showed no adherences with adjacent organs. The membrane showed a slight retraction and was surrounded by granulation tissue. Observations on 30th, 45th, 60th and 90th days showed an evolution of the process, resulting in a firm and reduced whitish area (Fig. 1). Similar aspects were described in the literature for the reconstruction of the cervical esophageal wall in dogs, abdominal wall in rabbits, as well as to the occlusion of hernial rings in dogs (7, 9). On the other hand, in this study it was observed adhesion of the implanted area with lung and liver in the an animal euthanized at the 30th day. No clinical signs were observed in this animal.

Microscopic examination showed a typical healing process with intense neovascularization, presence of inflammatory cells and formation of connective tissue and collagen. In the animals euthanized at the 15th day it was observed a profusion of inflammatory cells, both polymorphonuclear neutrophils and mononuclear cells; at 30th day inflammatory cells were mainly mononuclear; at 45th day, a decreased number of mononuclear cells were found in focal or diffuse distribution. In these times the presence of proliferating conjunctive tissue was profuse, with an increased amount of collagen. At 60th and 90th days, there was a clear parallel deposition of fibroblasts and collagen around the implanted membrane; the number of mononuclear cells was

reduced and the limit of the membrane with the scar tissue was clearly defined (Fig. 2). These results are similar to those previously reported in the literature (1, 6, 11).

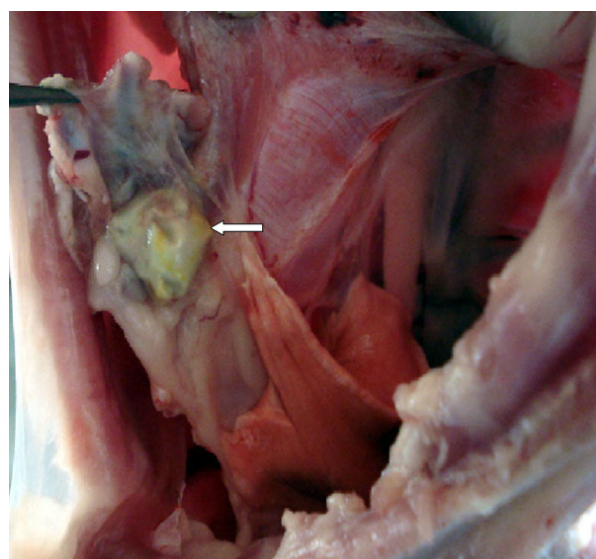


Figure 1. Latex membrane implanted in rabbit diaphragm. Thoracic face ninety days after surgery. There was a repair by fibrous conjunctive tissue. The membrane was easily removed (arrow).

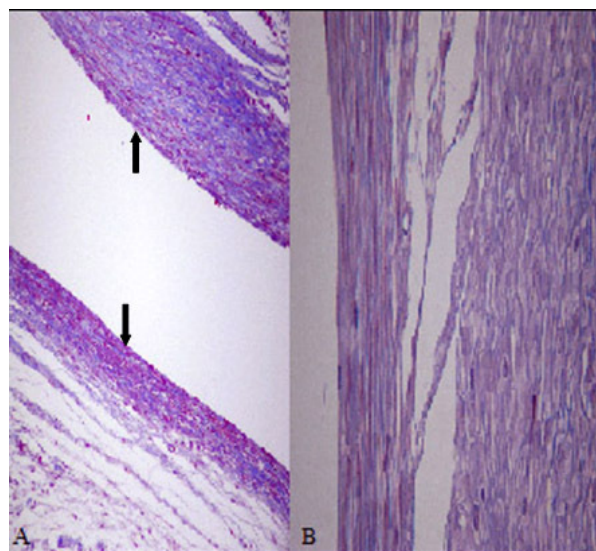


Figure 2. Microscopic aspect of the diaphragm ninety days after the latex membrane surgical implant. A - The membrane face contact produced a well defined surface in the fibrous tissue (arrows). TM, 20x. B - Aspect of the well oriented fibroblasts and collagen. TM, 100x.

The implanted latex membrane showed to be compatible with the recipient organism, once the healing process was appropriate and the membrane was easily removed as previously reported (1, 3, 8). The formation of fibrous connective tissue was sufficient to repair the diaphragm without loss of its function. Another positive aspect was no evidence of infection or rejection by the recipient organism (2).

In conclusion, based on the clinical and histopathologic evaluation, it was noted that the latex biomembrane showed excellent results for repair of the diaphragmatic defect, and it can be considered as a valid option for the treatment of this kind of injury. This material is natural, easy to handle, inexpensive and provides a blood supply in the implant site probably due to their angiogenic properties.

Acknowledgments

To CNPQ - Conselho Nacional de Desenvolvimento Científico e Tecnológico for a scholarship (Milena Friolani). To the Universidade de Marília for providing the rabbits. To Professor Joaquim Coutinho Netto for providing the latex membranes.

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