Crab cuticle membrane application for treatment of corneal lamellar laceration in rats: a preliminary study

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Crab cuticle membrane application for treatment of corneal lamellar laceration in rats: a preliminary study

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Abstract. Corneal lamellar laceration defined as a partial thickness injury to the cornea that may affect vision in various degree. Prompt management is needed to maintain integrity of the cornea and to avoid possible complications resulting from the injury. Material such as amniotic membrane had been widely use to promote reepithelization after corneal injury. Result from previous studies revealed that chitin, the most abundant substance in the cuticle layer of crab shell, is contribute to reepithelization after injury. Crab cuticle membrane preparation were made similar to amniotic membrane that is widely use in the management of corneal injury, therefore chitin might be sought as alternative material in the management of corneal injury which promote reepithelization. This study aimed to assess corneal epithelial proliferation profile and the number of keratocyte in rats after corneal lamellar laceration injury with and without application of crab cuticle membrane. Rats with corneal lamellar laceration injury were divided into groups treated with and without crab cuticle membrane application. Eyes were enucleated on the first and third days after treatment. Histologic preparations were made to assess corneal epithelial proliferation process and number of keratocyte in all groups. Cellular migration of more than 50 % were found in 66.7% of samples on 1 day after treatment and 61.1% of samples on the third days after treatment with crab cuticle membrane. The average number of keratocyte were 33.89 ± 5.27 cells/LPF on 1 day after treatment and 46.22 ± 9.55 cells/LPF on the third days after treatment with crab cuticle membrane. Epithelial proliferation after corneal lamellar laceration in rats treated with crab cuticle membrane application is faster and the number of keratocyte is more abundant compared to treatment without the application of crab cuticle membrane.
Keywords: Chitin; corneal lamellar laceration; crab cuticle membrane; epithelial proliferation; keratocyte.

1. Background

Ocular trauma is the most common cause of visit in the emergency department and inpatient unit. Approximately 800.000 patients admitted to the emergency department for eye surgery in The United States in 2000. Similar results were obtained from other studies, with reported incidence of three times higher in developing countries, especially in rural areas. This trauma causes decrease in visual function, hence proper management is required to prevent decreased quality of life [1-4].

Based on The Birmingham Eye Trauma Terminology (BETT), the classification of the corneal wound is divided into 2 groups, closed and open wound. Corneal laceration defined as wound to the entire thickness of the walls of cornea caused by sharp objects. A partial thickness injury of the eyeball is called lamellar laceration. Corneal wound management is very important because it can affect vision to various degree after wound healing process [3, 5].

Today the public interest in seafood and seafood products is increasing. One of the most popular seafood is crab. One part of the crab shell is the cuticle layer, with chitin as the most abundant substance. Ever since it was discovered 200 years ago, chitin is known as a natural cationic polymer that has been widely used as a topical dressing in wound management. Okamoto et al found a higher number of PMN cells in the chitin exposed group than in the control group. The formation of granulation tissue around the wound was also identified in the chitin exposed group. In wound healing process chitin promotes formation of collagen structure through its derivatives, glycosaminoglycan. Chitin has the ability to stimulates fibroblast proliferation as well as inflammatory cell migration in reepithelization process [6, 7].

Crab cuticle membrane preparation were made similar to amniotic membrane graft that has been widely used for the treatment of corneal injury as it can promote corneal reepithelization. The use of amniotic membrane as a temporary or permanent graft has become a common procedure in ocular surface reconstruction. The search for other synthetic and biocompatible materials that has the similar role as the amniotic membrane has been concerned and important as an alternative material for the management of corneal injury [7].

2. Materials and methods

This was an experimental study of corneal lamellar laceration injury in animal model treated with crab cuticle membrane in rats. This study was approved by the Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran. Twenty-four rats were selected and subdivided into four groups. The first and the third group were treated with artificial tears and ofloxacin eyedrop. The second and fourth group were treated with crab cuticle membrane application and ofloxacin eyedrop. Enucleation were performed after one day of treatment in the first and third group, and after three days of treatment in the second and fourth group. Corneal lamellar laceration injury was created using marked blade on the central cornea by 2 mm length and 0.1mm thickness.

The crab cuticle membrane used in this study had been processed by lyophilization drying techniques and sterilization by the National Nuclear Energy Agency of Indonesia Research Tissue Bank. Histologic preparations were made at Histology Department, Faculty of Medicine, Universitas Padjadjaran. Rats were obtained from the Laboratory of Clinical Pharmacology, Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran. Inclusion criteria were healthy white male BALB/c rats, age 10 weeks, body weight 250-350 grams. Exclusion criteria were rats that grew sick during the adaptation period, anatomical abnormalities in one or both eyes, and weight loss more than 10% during adaptation period. Drop out criteria were death during treatment period, inability to make histologic preparation from the objects and infection occurred after treatment.

Corneal tissue from enucleated eyes were preserved with formalin, made into paraffin block, cut axially and dyed with Hematoxylin eosin (HE). Histologic preparations were examined for epithelial proliferation and number of keratocytes between groups viewed under light microscope with 10 times magnification. Examination of histologic preparations of the corneal tissue was performed by mean of semi-quantitative assessment. Epithelialization were examined by the scale developed by Gal et al divided into 5 stages, 0: no cell migration; 1: cell migration <50%; 2: cell migration >50%; 3: bridging on excision wound; 4: total healing. Number of keratocytes were calculated based on the number of keratocyte observed per low power field (LPF) [8]. Each preparation was made from 10 corneal tissues randomly selected from objects included in the same group and the histologic examination were performed on 3 randomly selected preparations. Differences in semi-quantitative histologic examination between groups were analysed using Chi square test for categorical data and Mann-Whitney test for numerical data using SPSS 20.0 statistical program.

3. Results

Epithelial proliferation in groups treated with and without crab cuticle membrane application on the first and third days after treatment were shown in Table 1.

Table 1. Epithelial proliferation process in groups treated with and without crab cuticle membrane application on the first and third days after treatment

Assessment	Exposure		Frequency	Outcome (%)					P Value*
				0	1	2	3	4	
Epithelial Proliferation	Day 1	Group I	5	13.3	33.3	46.7	6.7	0	0.000
		Group II	6	0	0	5.60	66.7	27.8	
	Day 3	Group III	6	5.5	27.8	55.5	11.1	0	0.000
		Group IV	6	0	0	0	38.9	61.1	

Epithelial proliferation:
stage 0: no cell migration
stage 1: cell Migration <50%
stage 2: cell migration >50%
stage 3: bridging on an excision wound
stage 4: total healing
*Chi-Square test, significant if $p < 0.05$

On the first day after treatment, we found that in group treated with crab cuticle membrane, cellular migration of more than 50% were found in 66.7% of samples, whereas in group without crab cuticle membrane, cellular migration of less than 50% were found in 46.7% of samples. The difference between the two groups were found significant (p= 0.000). On the third days after treatment, we found that in group treated with crab cuticle membrane, total healing was found in 61.1% of samples, whereas in group without crab cuticle membrane, cellular migration of less than 50% were found in 55,.% of samples. The difference between the two groups were found significant (p = 0.000). Fig. 1 shows histologic view of epithelial proliferation in all groups on the first day and the third days after treatment.

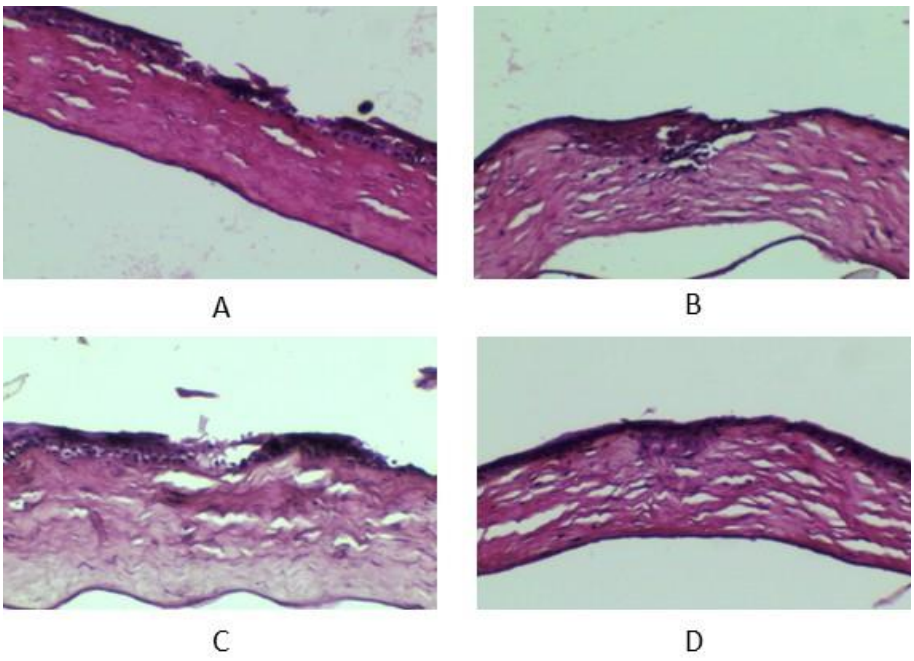

Figure 1. Histologic view of epithelial proliferation in all groups on the first day and the third days after treatment. A: group I, one day after treatment: B: Group II, one day after treatment; C: Group III, three days after treatment; D: Group IV, three days after treatment.

Number of keratocyte in groups treated with and without crab cuticle membrane application on the first and third days after treatment were shown in Table 2.

Table 2. Keratocyte count in groups treated with and without crab muticle membrane application on the first and third days after treatment

Assessment	Exposure		Frequency	Average (Cell /LPF)	P Value*
Keratocyte count	Day 1	Group I	5	15.47 ± 1.67	0.004
		Group II	6	33.89 ± 5.27	
	Day 3	Group III	6	24.50 ± 8.67	0.015
		Group IV	6	46.22 ± 9.55	

*Mann-Whitney test, significant

On the first day after treatment, we found that the average number of keratocyte in group treated with crab cuticle membrane were 33.89 ± 5.27 cells/LPF, whereas in group without crab cuticle membrane, the average number of keratocyte were 15.47 ± 1.67 cells/LPF. The difference between the two groups were found significant ($p = 0.004$). On the third days after treatment, we found that the average number of keratocyte in group treated with crab cuticle membrane were 46.22 ± 9.55 cells/LPF, whereas in group without crab cuticle membrane, the average number of keratocyte were 24.50 ± 8.67 cells/LPF. The difference between the two groups were found significant ($p = 0.015$).
if $p < 0.05$. Fig. 2 shows histologic view of keratocyte count in all groups on the first day and the third days after treatment.

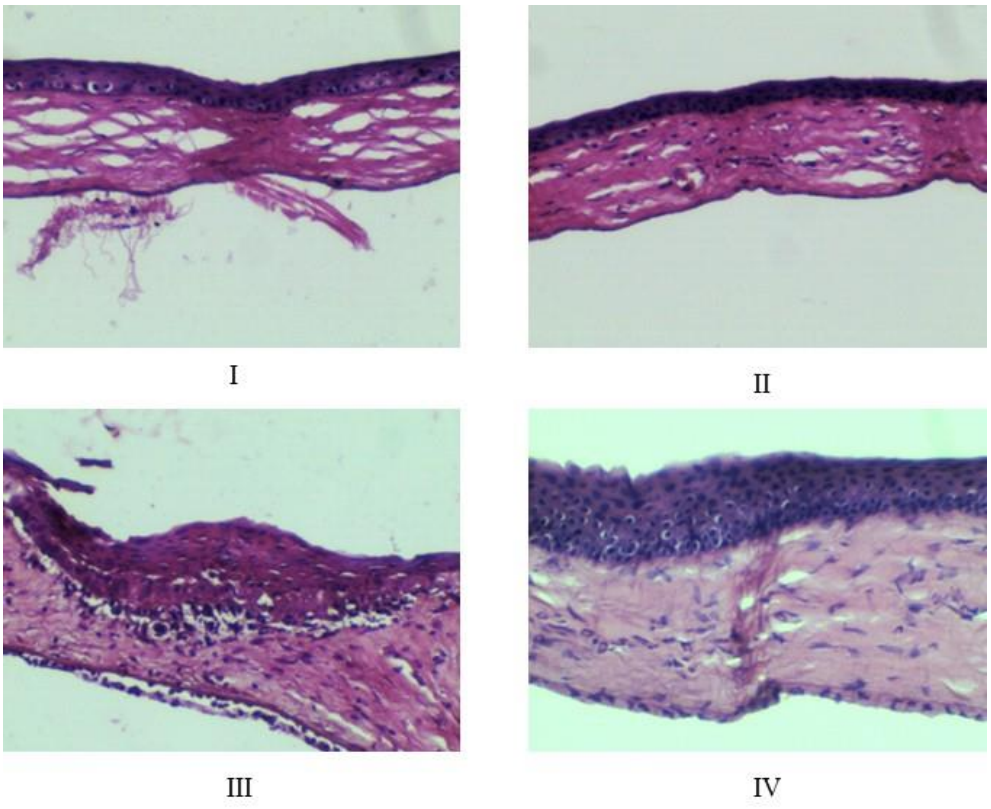

Figure 2. Histologic view of keratocyte counting all groups on the first day and the third days after treatment. In group IV (3 days after treatment with crab cuticle membrane) there were more abundant keratocyte counts compared to the other groups.

4. Discussion

One of the most frequent cause of visit to the emergency department is ocular injury. Corneal laceration is the most common form of the injury. Rapid and prompt diagnosis and management of corneal laceration is needed to prevent vision loss due to complications. One principle in the management of corneal injury is that one must pay attention to the morphology of the wound for this will determine

whether medical or surgical management is required to achieve good wound healing. Corneal wound healing process begins within the first 6 hours after trauma and reaches its peak in 6 days [3, 8-10]. Cuticle is an exoskeleton presents in crustacean made of calcified extracellular matrixes which contain chitin. Chitin has the ability to stimulate fibroblasts that is useful for reepithelization and to produce collagen fibres that plays role in the reconstruction of the wound tissue. Mori et al. reported that chitin and its derivatives accelerate cell proliferation in injured rats tissue due to the effects of angiogenesis and neutrophil migration. Kojima et al. reported that chitin can stimulate extracellular matrix-producing cells, which is an important factor for morphogenesis. The study also revealed that chitin has the ability to enhance the formation of collagen fibres, especially collagen types I, III, and IV. Chen et al. obtained chitin from culture of corneal epithelium in vitro, found an increase in the growth of epithelial cells without altering their endogenous characteristic [9-11].

This study was conducted on 24 white male rats BALB/c strain with treatment for corneal lamellar laceration. No toxicity was observed in all eyes throughout the treatment and enucleation process judging from the absence of redness and chemosis [12]. We evaluated epithelial proliferation process based on the scale developed by Gal et al. The process of epithelial proliferation in groups treated with crab cuticle membrane application is faster compared to groups without crab cuticle membrane application. This result is consistent with previous studies that stated that chitin has a role in reepithelization of wound tissue. In the wound healing process of the epithelial lining that takes about 24 hours after the trauma, glycoproteins and proteoglycans contained in the extracellular matrix have an anionic property that interact with chitin, thus chitin can increase the activity of the extracellular matrix that plays a role in wound tissue reepithelization. This substance also has the ability to stimulate fibroblast proliferation that will stimulate basal cells to perform epithelial cell replication [3, 13-15].

Assessment of the number of keratocyte showed statistically significant differences between group treated with and without crab cuticle membrane application ($p = <0.05$). This result is consistent with the previous study that FGF2 and EGF in chitin plays a role in tissue repair and increase keratinocyte differentiation. The wound healing process in the stromal layer of the cornea takes about 72 hours after the trauma. Chitin plays a role in increasing the activity of fibroblasts, thereby increasing keratocyte formation. Keratocyte form collagen fibres that help in the wound healing process [12, 14-17].

There are roles of collagen type IV, VII, XII, XV, XVII, XVIII on the formation of the basal membrane of the corneal epithelium. Stroma is composed of tight collagen fibre, in which type I and V collagen is the main constituent collagen fibers in the stroma. In the crab cuticle structure examined by the National Nuclear Energy Agency of Indonesia Research Tissue Bank, there is a collagen contained therein, however the specification of the collagen type is not yet known [3]. This study examined only the epithelial proliferation and keratocyte count, whereas the type of collagen and inflammatory cell activity that may affect the healing process of corneal injury was not examined. This is the limitation in this study.

5. Conclusions

The epithelial proliferation in the healing process of corneal lamellar laceration in rats treated with crab cuticle membrane application is faster compared to treatment without the application of crab cuticle membrane. The number of keratocyte in the healing process in rats treated with crab cuticle membrane application is more abundant compared to treatment without the application of crab cuticle membrane. Further study is needed to assess the effect of crab cuticle membrane application to collagen synthesis and inflammatory cell activity on the healing process of corneal injury, particularly immunohistochemically.

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