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Research Article

IN VIVO EVALUATION OF WOUND HEALING EFFICACY OF κ -CARRAGEENAN BLENDED POLYVINYL PYRROLIDINONE FILMS FOR WOUND DRESSING APPLICATIONS

[1, 2]Amruth P, [1, 2]Rosemol Jacob M, [1, 2]Jean Mary Joy, [1]R. Anandan, [1*]Suseela Mathew

[1]Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin-682029, Kerala, India
[2]Faculty of Marine Sciences, Cochin University of Science and Technology, Cochin-682022, Kerala, India

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ABSTRACT

The requisite for novel potential dressings has emerged as a consequence of the incidences of injuries caused due to second intention and expensive treatment costs for the management of them. New generation dressings are capable to accelerate proliferation of cells and regeneration of tissue despite of the protective role to the wounds. Moreover, patient compliance persists as an integral factor with regard to the fact that they create no damage to the newly regenerated tissue on removal of the same. In the current study, we developed a κ- carrageenan film dressing, which is capable to accelerate tissue repair and aids its removal by external stimulus without disturbing the regenerated tissue. The dressing is capable to absorb excessexudate from the wound by maintaining hydrationto wound bed. The medical dressing was evaluated in male Wistar rats by generating a pocket wound. The dressings were implanted in incised pocket wound for 16 days. Histological analyses demonstrated that the dressing inserted on the injured area have showed minimal level of inflammatory events and generated denser connective tissue on comparison to wounds devoid of dressings. The injured site sheltered with the dressing demonstrated re-epithelization and angiogenesis to promote wound closure.

Keywords: Seaweed, Carrageenan dressing film, Wound closure, Wound healing.

INTRODUCTION

Globally, there are a significant number of reported cases of chronic wounds each year. The management of these wounds requires substantial financial resources, estimated to be in the thousands of US dollars per treatment (West *et al*., 2007). Chronic wounds encompass a variety of conditions such as complicated burns, diabetes-related wounds, pressure ulcers, and venous ulcers, which typically take a longer time to heal compared to other types of wounds (Ignacio *et al*., 2011; Izadi *et al*., 2005). Wounds that have the potential to heal through secondary intention are quite common where the presence of granulation tissue is visible.

Given the high incidence rate, cost implications, and impact on individuals, numerous medical devices have been developed to facilitate wound protection and healing (Draye *et al*., 1998; Cho *et al*., 1999). These devices are designed with porosity as a crucial feature to ensure proper retention of exudate from the wound bed (Ulubayram *et al*., 2001). Although these medical devices effectively protect the injured site and promote accelerated wound closure, some types of dressings can cause adhesion between the newly formed tissue and the dressing itself upon application (Edahiro *et al*., 2005). Consequently, the removal of such dressings can lead to tissue damage and discomfort for the patients. In the current study; we aimed to evaluate the rate of wound closure in an incised area by applying a carrageenan transparent film dressing.

MATERIALS AND METHODS

Preparation of carrageenan transparent film dressing

The carrageenan transparent film dressing was prepared using the solvent casted method. A solution containing 2%

*Corresponding Author: Dr. Suseela Mathew, Principal Scientist & Head of Division Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin-682029, Kerala, India. Email: suseela1962@gmail.com, Mobile: +91 94462189558.

(w/v) carrageenan (KC), polyvinyl pyrrolidone (PVP) and 0.5% (v/v) glycerol was created, which served as the film forming solution (FFS). To obtain a transparent solution, the polymers were dissolved in deionized water while continuously stirring at 1400 rpm at a temperature of 80°C. The FFS was then sonicated for 30 minutes at 80°C and left at room temperature until any air bubbles disappeared. For the production of the carrageenan transparent film, the gel was poured into a polypropylene plate with a diameter of 60 mm. The gel-filled plate was then subjected to oven drying for 8 hours. This process allowed for the development of the transparent film dressing. The film, after undergoing oven drying, was stored in sterile bags for further use.

In vivo wound healing study

The carrageenan transparent film which was prepared earlier was used in a pocket wound created in Male Wistar albino rats. Prior to the study, the animals (8 weeks old, with a mean body weight of 175-200 g) were acclimatized for 7 days and housed in polypropylene cages under standard environmental conditions, including a temperature of 28±2°C, humidity ranging from 60% to 70%, and a 12-hour light/dark cycle. The animals were provided with a standard diet and had access to water ad libitum. The experimental setup consisted of three groups of animals, each containing six rats. The first group served as the control group, where the rats had a wound without any treatment. The second group received treatment with the carrageenan transparent film dressing, while the third group was treated with a commercial dressing. To initiate the experiment, the animals were anesthetized by intraperitoneal administration of Ketamine (75 mg/kg body weight) and Xylazine (10 mg/kg body weight). The dorsal portion of the rats was shaved and sterilized using a 70% ethanol solution (Sumayya and Muraleedhara Kurup, 2018). A sterilized surgical blade was used to create an incision of approximately 15 mm, and a subcutaneous pouch was formed on one side of the incision. A piece of the scaffold material, measuring approximately 10 mm^2, was inserted into the pocket and sutured using a non-absorbable silk thread. On the 16th day after the implantation, the animals were sacrificed, and skin tissues along with the surrounding implant area were collected for histopathology analysis. The research studies conducted in this experiment were approved by the Animal Ethics Committee of ICAR-Central Institute of Fisheries Technology, Kerala, India (Ethical Approval Number: CIFT/B&N/IAEC/2020-1(3)). The guidelines of the Institutional Animal Ethical Committee (IAEC) were followed throughout the study to ensure the welfare and ethical treatment of the animals involved.

Wound closure measurement

Throughout the duration of the study, the wounds treated with different dressings were closely monitored to evaluate the integrity of the dressings and observe the behaviour of the animals. Photographs of all the animals were taken using an iPhone XR in the standard prone positions to assess the scars and compare the treated groups. At specific time points, namely days 0, 3, 7, 12 and 16 all wounds from each group of six animals were assessed and photographed three times. To calculate the wound closure rate, the following equation was employed:

$$\text{Wound closure rate in \%} = ((Wo - Wt) / Wo) \times 100$$

Where: Wo represents the initial wound area, Wt denotes the wound area at a specific time point, represented as "t". This equation allowed for the quantification of the percentage of wound closure over time, providing insights into the effectiveness of the different dressings in promoting wound healing.

Histopathology Examination

At the end of the study period, the rats were humanely euthanized using a CO_2 chamber with a flow rate of 3 ml/min for 5-10 minutes. This method ensures a painless and rapid euthanasia. The skin was carefully and aseptically removed from the rats and fixed in a 10% buffered formalin fixative overnight. To prepare the skin samples for analysis, sections of 5 µm thickness were embedded in paraffin wax and stained with hematoxylin and eosin (H&E).

The stained slides were then examined under a light microscope to assess the extent of damage and evaluate the healing process. Both healthy and injured skin sections were analyzed to compare the effects of the carrageenan transparent film dressing. The evaluation involved observing and analyzing the tissue structures and characteristics. Images of the skin sections were captured using a light microscope equipped with a digital camera, specifically an Olympus microscope. This allowed for a detailed examination and documentation of the histopathological changes in the tissue caused by the treatment with the carrageenan transparent film dressing.

Statistics

Using one-way ANOVA, statistical analysis was performed, followed by Tukey's multiple comparisons test, by using Prism version 6.00 (GraphPad Software, La Jolla, California, USA). The statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

All treatment groups showed wound closure within 16 days. The wounds treated with the carrageenan transparent film dressing exhibited faster healing compared to those treated with the commercial dressing. Figure 2 illustrates the wound closure rate (WCR) over time for the groups treated with the carrageenan transparent film dressing. The wound closure rate was measured at 3, 7, 12, and 16 days, as shown in Figure 2. The carrageenan transparent film dressing-treated group demonstrated a significantly higher wound closure rate compared to the control and

commercial dressing-treated groups. The carrageenan transparent film dressing remained intact and did not adhere to the wound during the application period. It was also easy to remove without causing any skin damage. By day 16, almost complete healing of the wounds was observed in the groups treated with the carrageenan transparent film dressing (90.5%) and the commercial dressing-treated groups (88.6%), as depicted in Figure 2. The wounds treated with the carrageenan transparent film dressing showed better tissue quality and less scarring. During the wound healing process, carrageenan gradually breaks down, releasing d-galacto pyranose. This compound promotes fibroblast proliferation and facilitates the organized deposition of collagen. It also stimulates the synthesis of natural hyaluronic acid at the wound site, aiding in the healing process (Jayakumar *et al*., 2011). In the case of the carrageenan transparent film dressing and commercial dressing, no degradation was observed. The biocompatibility of the implanted carrageenan transparent film dressing material was evaluated over a period of 60 days, although the results of this study are not included in this report.

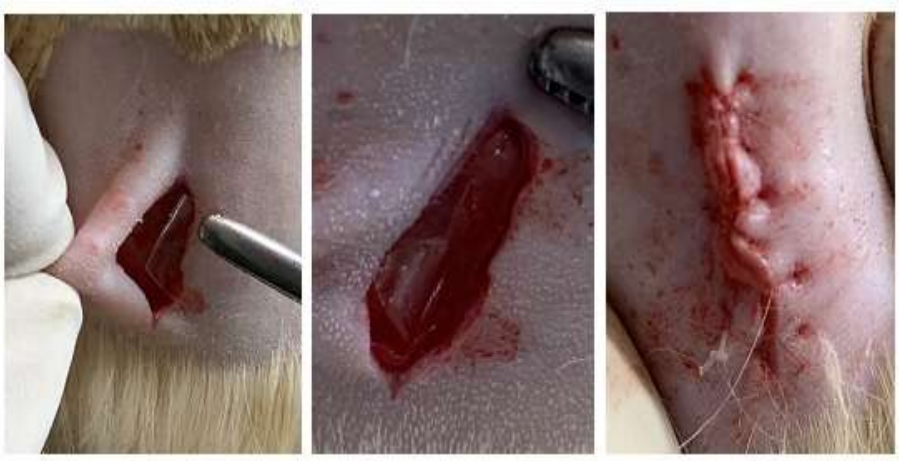

Figure 1. Implantation of the carrageenan transparent film dressing in the incised pocket wound (a) Pocket wound (b) carrageenan transparent film (c) implantation of the wound dressing.

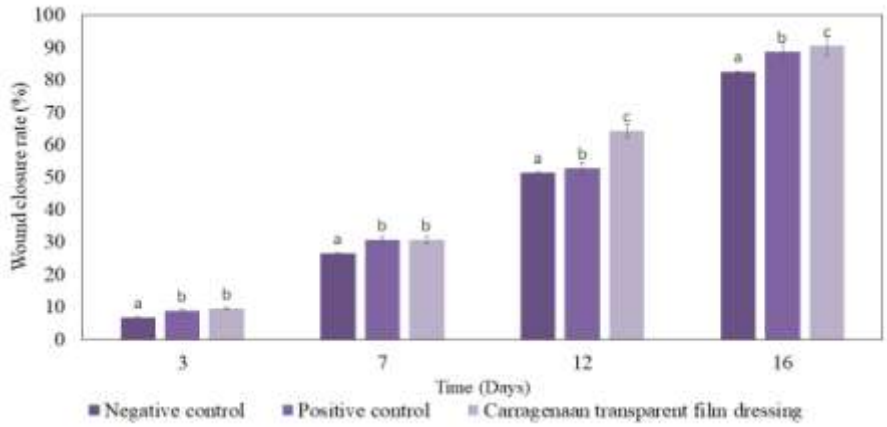

Figure 2. (a) Appearance of wound treated with carrageenan transparent film dressing. (b) Different superscripts (a-c) indicate a significant difference among difference days of treatment at $p < 0.05$.

The histological evaluation reports provide valuable information regarding the condition of the injury. On the 16th day of the study, the wound treated with the carrageenan transparent film dressing exhibited epidermal thickening, and the surface of the wound appeared to be covered with new epithelium, as depicted in Figure 3. The presence of skin regeneration was prominent, with well-developed dermis and epidermis. Matured fibrous tissue proliferation was observed in the dermal region. In the negative control group, the wound shown in Figure 3 demonstrated loose granulation tissue with necrotic patches and sparse blood capillary development. In the groups treated with the commercial dressing, inflammatory cell infiltration was noted, and the epidermis and dermis showed loosely arranged, immature fibrous tissue formation. The histological observations indicate positive signs of wound healing in the group treated with the carrageenan absorbent film dressing. Consequently, the healing process was notably better in the carrageenan transparent film dressing-treated wounds compared to the wounds treated with the commercial dressing. The functionality of the carrageenan transparent film dressing is illustrated schematically in Table 1 and Figure 2.

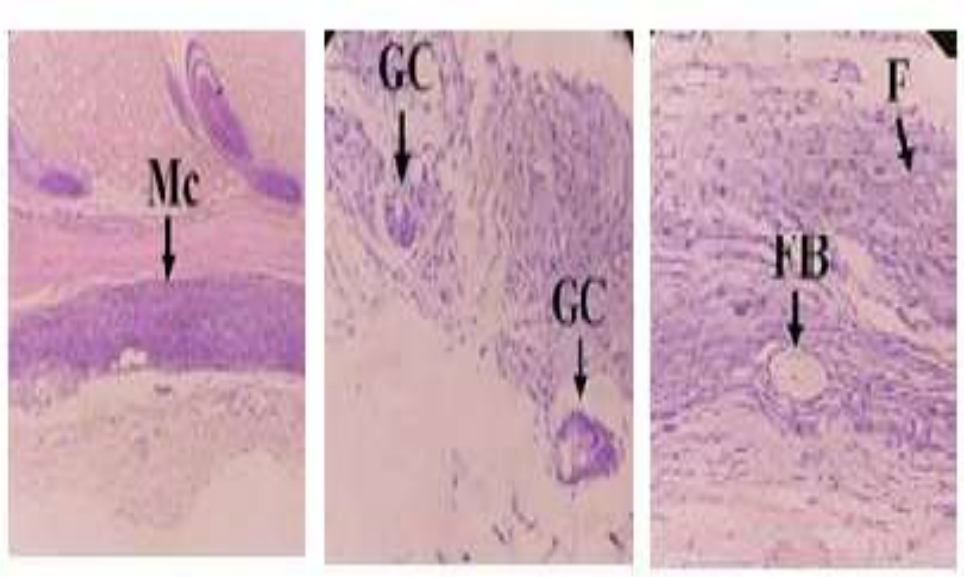

Figure 3. Hematoxylin-and eosin-stained sections of wound tissues treated with carrageenan transparent film dressing. The markings are as follows, Mc- Macrophages. Gc-Giant cells, FB- Foreign body, F- Fibrosis.

Table 1. Histopathological findings of Hematoxylin-Eosin Stain.

Layers	Observation Points	Assessments	
		Control	Carrageenan transparent film dressing
Epidermal Layers - Cornified Cell Layer	Keratin filament	Hyperkeratosis	Keratin filaments are normal in structure
Epidermal Layers - Granular Cell Layer	Keratiocyte Keratin granules	Very less, scattered Less	More, oriented More
Epidermal Layers -Spinous Cell Layer	Cuboidal cell to flattened squamous cell	Less in number	More in number
Basal Layer	Single layer of cuboidal or low columnar cell	Not distinguishable	Single cell layer
Dermal Layer	Chronic inflammatory cell Fibroblast	Less Less	More More

CONCLUSION

The current experimental study on wound healing demonstrates that the carrageenan transparent film dressing promotes efficient hemostasis, effective healing, and re-epithelialization of the wound. The histopathological observations suggest that the cells beneath the carrageenan transparent film dressing have stimulated the regeneration of skin cell layers and contributed to the restoration of tissue architecture. The developed dressing has shown promising results in both *in vitro* and *in vivo* wound healing studies conducted on experimental animals. However, further clinical studies are necessary to provide more comprehensive evaluations of the carrageenan transparent film dressing. These studies will help to determine its efficacy and safety in human patients, which is an important step towards potential clinical applications.

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