Journal of Dentistry and Dental Practices
ISSN: 2689-5994

Academic Strive
Researcher's perception is our reality

Research Article *Volume 7 Issue 1*

Formation of Rat Gingival Epithelium after Application of Carrageenan-Based Oral Wound Dressings

Indahyani DE*[1], Barid I[1], Praharani D[2] and Probasari N[2]

[1]Department of Oral Biology Faculty of Dentistry, University of Jember, Indonesia
[2]Department of Periodontic Faculty of Dentistry, University of Jember, Indonesia

***Corresponding author:** Didin Erma Indahyani, Department of Oral Biology Faculty of Dentistry, University of Jember, Jl Kalimantan no 37 Jember, East Java, Indonesia, Email: didinermae.fkg@unej.ac.id

Received Date: June 05, 2025; **Published Date:** June 13, 2025; DOI: 10.63235/DDPJ.180106

Abstract

A wound dressing is necessary to promote postoperative wound healing by preventing plaque buildup and bacterial invasion. Dressing made from hydrogel maintains wound moisture and is antibacterial and flexible/elastic. Applying dressing as a wound cover can stimulate the acceleration of healing. The formation of epithelium in wounds is one of the indicators of wound healing. Red algae contain carrageenan, a polysaccharide of hydrogel nature that can potentially be a wound dressing ingredient in the oral cavity. This study aimed to analyze hydrogel oral wound dressing based on red algae carrageenan on epithelial formation. Method. Male Wistar rats aged 2-3 months were performed horizontal incisions 2 mm long in the 0.5 mm area of the gingival edge of the midline region of the central incisive of the lower jaw. The extraction of kappa carrageenan was synthesized into a wound dressing and applied to cover the wound immediately after the incision. The negative control group is applied with dressing Erbemowcare®. For the positive control group, the wound lets without wound coverings. Epithelial formation is observed in histological preparations. The epithelium formation between the rats with Erbemowcare® oral. Wounds were covered with wound dressings (carrageenan or commercial), the formation of the epithelium was visible on the third day. In contrast, epithelium formation is visible in wounds without dressing on the seventh day. The epithelial thickness score in wounds with carrageenan-based and commercial dressings did not differ significantly ($P<0.05$). Conclusion. It was concluded that the application of oral wound dressing based on hydrogel from carrageenan was able to accelerate the formation of epithelium in the wounds of the oral cavity.

Keywords: Red Algae; Carrageenan Iota and Kappa; Wound Healing; Periodontal Dressing

Abbreviations

HE: Hematoxilin Eosin; MMP: Matrix Metalloproteinase; ECM: Extracellular Matrix; PEG: Polyethylene Glycol; PVA: Polyvinyl Alcohol; TGF-β: Transforming Growth Factor-Beta.

Introduction

The healing of open wounds after periodontal surgery in the oral cavity is often hampered by the accumulation of plaque bacteria and the burden of mastication. Oral wound

dressings are needed as a physical barrier [1], to prevent bleeding, the formation of granulation tissue and promote healing [2]. The use of dressings should consider the general health of the patient, aetiology and the phase of the wound. Modern wound dressings must be multifunctional and have minimum adhesion to the wound surface, adsorption effect of removing exudate and toxic compounds, hemostatic, involved in gas exchange, providing moisture, elastic, antimicrobial, thermal insulation, biocompatible, easy to decompose after use, strong, efficient and quite cheap [3]. There are no oral wound dressing products that meet these requirements. The antibacterial ability of the dressing material is usually due to the presence of additive agents [4].

Hydrogels are hydrophilic polymer macromolecules in cross-linked networks with high diffusion power [5]. These properties control the wound surface's hydration and absorption of exudate and provide moisture that facilitates wound healing [6]. Polysaccharides extracted from red algae are capable of forming hydrogels. K-carrageenan is a linear polysaccharide in red algae, composed of 1,3-linked -D- and 1,4-linked -D-galactose substituted with three sulphate groups per disaccharide unit [7]. The high sulphate content is an antioxidant [8]. Carrageenan has been used as an antibacterial, anti-tumour, and tissue engineering. As a dressing, it improves the healing process by affecting the wound repair stage [3]. The use of hydrogel as a wound dressing is based on its antioxidant properties that can eliminate excess reactive oxygen species in chronic wounds and reduce oxidative stress, thereby stabilizing the microenvironment of the wound, improving collagen synthesis and re-epithelization, and reducing the pH value of the wound to speed up healing and reduce infection [9].

The epithelium is one of the important main characters in the gingival structure that protects the underlying tissues from mechanical trauma, microorganisms, and chemicals. In addition, the gingival epithelium also has a complex biological function in immune defence and periodontal tissue homeostasis [10]. One of the leading indicators of the success of the wound healing process in the oral cavity is the reshaping of the gingival epithelium, which plays a vital role in the integrity and protection of the tissues around the teeth [11]. The purpose of this study was to analyze gingiva epithelial formation after the application of carrageenan-based oral wound hydrogel dressing in rats.

Material and Methods

Research Materials

Carrageenan extract from red algae (Kaphaphicus. alvarezii) was obtained from the oral biology laboratory of the University of Jember, and the procedures and test results followed the research of Indahyani DE, et al. [12], carbomer, gelatine, propylene glycol, glycerol, TEA, ketamine, Erbemowcare®, *xylazine, male Wistar rats were purchased from the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University,*

Research Groups and Designs

This research procedure follows the Experimental Animal Research Ethics Commission. It has been approved by the Ethics Research Committee no 1648/UN25.8/KEPK/DL/2022. The study was conducted on 36 male Wistar rats aged 2-3 months and divided into three groups. The first group is positive control (wound was left open), the second group is negative control (oral wound was covered using wound dressing Erbemowcare®), *and the third group is oral wound was covered using wound dressing kappa carrageenan). Each group was divided into three sub-groups to be observed on days 1, 3 and 7.*

Producing of hydrogel wound dressing

The composition of oral wound dressing is based on the modified research of Struck MB, et al. [13]. In brief, it is as follows: The composition is 0.375 g of carbomer and 0.55 g of gelatin mixed with distilled water and left for 24 hours. Then homogenization was carried out with a hand mixer and 2 g of propylene glycol, 12.5 g of glycerol and 1 g of TEA. The mixture was homogenized until it was mixed. Next, distilled water was added until the total weight was 30 g. To make a kappa hydrogel, 0.375 g of carrageenan kappa was added to the composition. The mixture was then poured into Petridis with a thickness of 2 mm. Put in the oven at 600C for 4 hours.

Treatment of Experimental Animals

The rats that were adopted were separated into groups. Rats were injected peritoneally with ketamine 10% mg/kg body weight (BB) and 2% xylazine at a 0.1 ml/100 g BB dose [13]. Asepsis was performed on the gingiva in the left region of the lower jaw central incisor. Then, an incision of 2 mm was made horizontally with a depth of 0.5 mm using a scalpel. The wound is closed immediately using a wound dressing, according to its respective group. On the first, third, and seventh days after the procedure, the mice were killed using a predetermined procedure. The tissue was taken and separated from the others to make histological preparations using hematoxylin and eosin staining. The epithelium formation is observed under a light microscope, and its thickness is measured. The tissue was taken, separated from the others, and put in a pot containing a 10% neutral formalin buffer to be fixed. After 24 hours, the tissue is prepared for histological preparation with hematoxilin eosin (HE) staining. The epithelium formation is observed under a light microscope, and its thickness is measured. The thickness of

the gingival epithelium that forms in the wound area was measured using a digital micrometre attached to a light microscope and connected to a USB with a real-time image display. Observations were made at 100x magnification, from the basal layer to the stratum corneum perpendicularly, starting from the new epithelium at the edge of the wound. One observer measures thickness at the two thickest and thinnest points. The final result was calculated from the average of the two points.

Data Analysis

Histological data analysis was carried out on the thickness of their epithelial formation. The data obtained was tabulated with SD ±2 and analyzed with two-way ANOVA followed by an LSD test with a confidence level of 95%.

Results and Discussion

The wound-healing process in the mucosa of the oral cavity is a complex and dynamic series consisting of inflammatory, proliferation, and remodelling phases. One of the important indicators of successful healing is the reformation of the epithelial layer (reepithelialization). In this study, carrageenan-based wound dressings showed typical epithelial progressivity, which differed between the 1st, third, and seventh days (Table 1).

No	Group	1 day	3 days	7 days
1	Control without dressing	0^{a}	$1,4 \pm 40^{c}$	$8,1 \pm 3,5^{b}$
2	Sheet Gel hydrogel Erbemowcare	0^{a}	$8,2 \pm 02^{b}$	$15,4 \pm 34^{d}$
3	Kappa carrageenan	0^{a}	$4,30 \pm 54^{c}$	$13, 26 \pm 41^{d}$

Table 1: Epithelial formation thickness (***μm***).

Remarks: Epithelial thickness value is expressed as mean ± SD. Statistical analysis using two-track Anova with a confidence level (95%). Different letters in each number indicate a significant difference (P<0.05).

On the first day after injury, the formation of new epithelium has not been seen in all groups. Statistically, on the first day, there was no significant difference (P>0.05) between the groups in the formation of the epithelium. This is closely related to the dominance of the acute inflammatory phase, where the infiltration of immune cells such as neutrophils and macrophages still dominates the wound area [14]. Phagocytosis activity by these cells and the release of inflammatory mediators such as interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF-α), and ROS (Reactive Oxygen Species) are the primary focus of the body in eliminating pathogens and tissue debris [15]. The initial event after the homeostasis event is the infiltration of inflammatory cells in the wound area. The peak of this inflammatory response is 24 – 48 hours post-injury [16]. At the beginning of this inflammatory phase, cytokines are present, and the reduction of blood vessels and the formation of fibroblasts begin [17]. In addition, there is also an increase in neutrophil cell migration to degrade damaged matrix regions by secreting proteases, namely matrix metalloproteinase (MMP) [18]. Neutrophils also initiate a cascade of cytokine secretion and growth factors to recruit other immune cell cells, including monocytes, which aid in the formation of the epithelium. In long or failed wound-healing cases, the neutrophils persist and create chronic wounds by producing proteases [19].

On the first day after injury, wound dressing (carrageenan or synthetic hydrogel) plays an important role in preparing the wound environment so that the epithelialization process can run faster. Its function is more emphasized to maintain moisture so that evaporation can be prevented and support the viability of migrating cells [20]. Dressing also reduces microbial contamination that acts as a physical and chemical barrier [21]. In addition, dressing stabilizes the wound's microenvironment by maintaining pH and temperature. Dressing protection on the wound will prevent re-trauma from food, chewing movements and contamination of salivary fluid [22].

On the third day, the group of rats with wound dressings microscopically began to see epithelium formation. The thickness of this epithelial formation was significantly different (p<0.05) between the first, second and third days. The thickness of the epithelium also differed significantly (p<0.05) between the group without dressing and the group applied with dressing (Table 1). The beginning of the formation of this epithelium indicates a transition from the inflammatory phase to the proliferative phase. Basal keratinocytes from the edge of the wound begin to undergo activation, proliferation, and migration to cover the wound surface on this third day [23]. Another study showed that monocytes migrate 48 to 72 hours post-injury on the third day. Monocytes will differentiate into macrophages. Macrophages become the primary cells during this healing phase [24]. Macrophages will secrete cytokines, including interleukin-1, interleukin 6, fibroblast growth factor, platelet-derived growth factor, epidermal growth factor and TGF-β which play a role in the migration of keratocyte cells and fibroblasts in the wound. Keratinocyte cells from the edge of the wound are activated due to stimuli from cytokines and growth factors released during the previous inflammatory phase, such as EGF, KGF (Keratinocyte Growth Factor), and TGF-α. These cells undergo phenotypic changes to become more migratory and begin to crawl into the wound area by utilizing fibrin matrix and granulation tissue as substrates.

This process is known as epithelial tongue formation [25]. Histologically, on the third day, a thin layer of one to two epithelial cells usually begins to appear on the surface of the wound (Figure 1). Its morphology is elongated and flattened cells. On the third day, neutrophil infiltration decreases, and active fibroblasts appear in the tissues underneath, signalling a transition from the inflammatory to the proliferative phase.

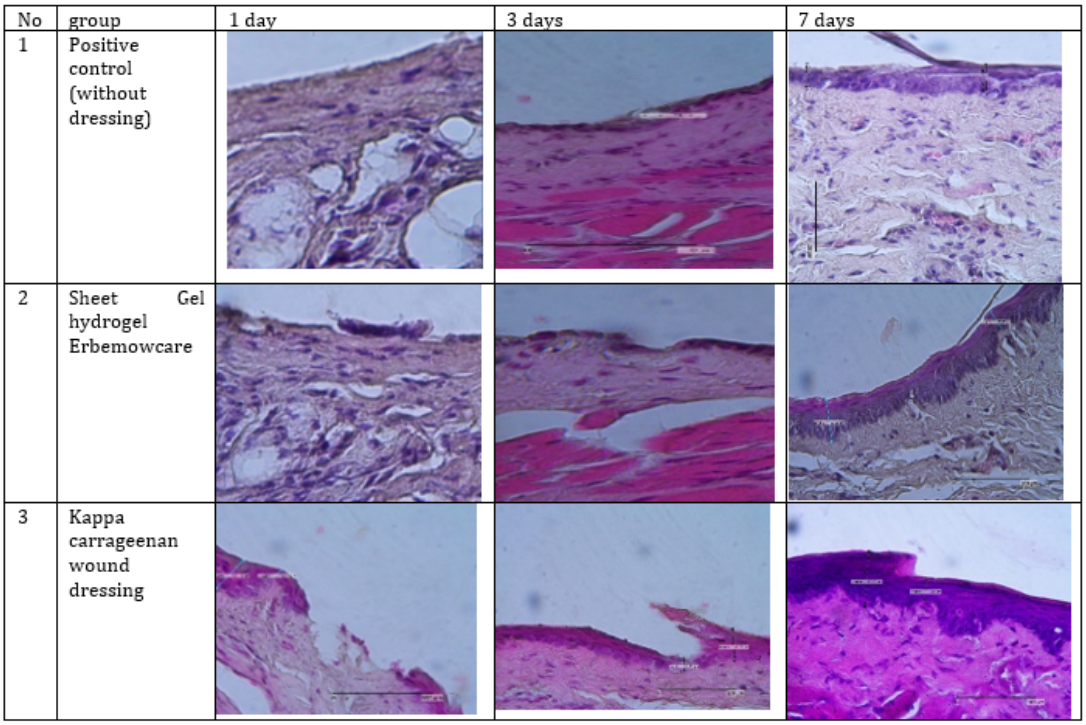

Figure 1: The histological picture with 100 x magnification.

In contrast to the group of mice without wound dressings, the epithelial formation was very thin. It was not yet clearly visible (Figure 1). In wounds that were not given dressings (without dressing), the reepithelialization process was delayed or slowed down so that there was no histological apparition of epithelium on the third day. This is because the wound surface is not protected and is directly exposed to the environment of the oral cavity with saliva and a diversity of bacteria. Proteolytic enzymes, such as amylase and lysozyme in saliva, can disrupt the stability of the initial fibrin matrix needed for epithelial cell migration substrates [26]. In addition, mechanical exposure to the oral cavity's food, tongue, and muscle movements can lead to re-traumatization. Unstable wound microenvironments such as fluctuations in temperature, pH, and humidity cause the wound microenvironment to become unstable, worsening the regenerative process. Without bandages, the wound becomes dry due to the evaporation of tissue and saliva fluids. This condition causes the death of migrant epithelial cells that are sensitive to desiccation (dryness), crust production (scrab) or dry fibrin layers that inhibit keratinocyte migration [20]. Previous research has shown that wound moisture is important in accelerating reepithelialization. Wounds that are kept moist close faster than open wounds [27]. Unprotected wounds undergo more intense and prolonged inflammation, which is characterized by more infiltration of neutrophils and macrophages on day 3, producing more ROS and proteolytic enzymes that can damage migratory cells and ECM, excessive production of inflammatory mediators, such as IL-1β, TNF-α, which slows the transition to the proliferative phase and secondary tissue damage due to uncontrolled inflammation, which delays the recruitment and proliferation of basal keratinocytes. Reepithelialization requires transient substrates that support epithelial cell movement, such as fibrin matrix, fibronectin, and type III collagen [28]. This matrix is rapidly degraded in open wounds by environmental exposure and proteolytic enzymes. The adhesion of keratinocytes is disrupted so that the migration of epithelial cells from the edge of the wound becomes inefficient [23]. Histologically, in the group of rats without wound dressing, the wound surface was still dominated by fibrin exudate, inflammatory cells (neutrophils), and cellular debris. The formation of migratory epithelial cells has not been seen to cover the wound's surface, or when it exists only in very small numbers and has not formed a continuous layer. No signs of epithelial tongue formation indicate that

early keratinocyte migration has been ineffective [29].

The dressing application provides a microenvironment that supports the epithelial formation process by maintaining the wound's moisture. The hydrophilic properties of the dressing materials of both carrageenan and factory-made dressings will retain moisture in the wound area, which prevents desiccation and facilitates the optimal migration of keratinocytes. Carrageenan's antioxidant properties help to lower local oxidative stress and epithelial cell activation [30]. Hydrogel materials also resemble an extracellular matrix (ECM) that provides keratinocyte adhesion facilities and accelerates migration [31].

In mucosal wounds of the oral cavity, wound dressing has a key role in creating a microenvironment conducive to the healing process, including reepithelialization. Synthetic hydrogel dressings, such as those based on polyethylene glycol (PEG), polyvinyl alcohol (PVA), or polyacrylamide, have been shown to accelerate and strengthen the formation of epitheliums that can be even thicker than natural dressings such as carrageenan. Based on histological observations on days 3 and 7, the epithelium formed in the wound with synthetic hydrogels tends to be thicker, consisting of 3–4 layers of non-keratin squamous epithelial cells. This suggests that the proliferation and differentiation of keratinocytes take place more actively. This thickness reflects not only the speed of epithelialization but also the possibility of an increased proliferative response stimulated by the physical and chemical environment of the hydrogel. In addition, the very high moisture retention of carrageenan creates an ideal environment for cell proliferation [32]. The ability of the hydrogel to retain local bioactives, such as endogenous EGF or KGF, strengthens epithelial stimulation. The synthetic hydrogel properties are more cohesive and adhere more to the wound surface, keeping the wound area perfectly closed and preventing desiccation or contamination. This cohesive property shows that synthetic hydrogel dressings are stickier than carrageenan-based dressings. The high adhesion properties ensure close contact between the dressing and the wound surface, creating a biological seal that prevents contamination and supports reepithelialization and can maintain the stability of temperature, humidity, and pH in the wound area and accelerate the activation and migration of keratinocytes [33]. However, an attachment that is too high poses a risk of trauma when changing dressings due to high adhesion. For example, it can cause damage to the epithelial that is being formed if not removed carefully [34]. The sticky nature can also resist debris or exudate, which, if not appropriately cleaned, can slow down the remodelling [35].

As a sulphated polysaccharide, Carrageenanan interacts with extracellular matrix proteins such as fibronectin and laminin and supports the adhesion and migration of epithelial cells [36]. The gel properties of carrageenan also help maintain a moist wound-healing environment that has been shown to support the proliferation of epithelial cells and fibroblasts [37]. In addition, there are reports that carrageenan can stimulate the release of growth factors such as epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β), which play an important role in reepithelialization [38]. Therefore, carrageenan-based dressing formed an epithelium on the third day of the group. On the seventh day, the epithelial layer appeared thicker and better composed than the group without dressing application. This indicates that the process of keratinocyte differentiation has taken place. This new epithelial layer begins to show a structure that resembles the normal epithelium of the oral mucosa, accompanied by the restoration of the connective tissue structure underneath. The difference with the group that was not applied with wound dressings was seen in the degree of maturation and smaller thickness of the epithelium.

Carrageenan is a biocompatible and biodegradable biomaterial that provides mechanical and biochemical support for tissue regeneration. In this context, it acts not only as a wound protector but also as a scaffold that supports cell adhesion, proliferation, and the migration of keratinocytes and fibroblasts [39]. Decreased inflammatory cell infiltration and increased fibroblast activity also contribute to the stability and maturation of new epithelium. Studies show that biomaterials such as carrageenan that are biocompatible and non-toxic can increase the expression of epithelial cell proliferation-related genes, such as cyclin D1, as well as stimulate mild angiogenesis that is important for the nutrition of newly formed epithelial cells [40]. Indahyani DE, et al. [12] stated that carrageenan-based dressings stimulate the proliferation of fibroblast cells in vitro.

The thickness of the epithelial growth was slightly thinner than in the group with a factory-made hydrogel dressing. In wound dressings, Kappa carrageenan (group 3) causes mechanical differences in properties with factory-made dressings. The characteristics of carrageenan-based wound dressings are rigid, hard, and brittle. The surface structure is smoother, and the viscosity is high and not so sticky. This change in properties is due to the kappa karageenan structure, which is composed of anionic polygalactane sulphate with long linear chains that are alternately located α-1,3 D-galactose and β-1,4 3,6-anhydro-galactose (3,6-AG) and its sulphate esters. In general, carrageenan will form a three-dimensional network of double helixes through the cross-linking of adjacent sulphate groups. After the gel cools, the coil transitions, so the helix and the aggregation of the helix form thermotropic and ionotropic, resulting in the carrageenan being brittle and the development ratio being high. The mechanical stability is poor [41]. Generally,

carrageenan, in the form of sodium, potassium and calcium salts or both, functions to provide stability. The sodium form is soluble, and potassium is less soluble in solubility. The salt will interact with the three-dimensional gel, which causes the gel to appear transparent or cloudy, rigid, elastic, hard and stable to heat or thermally reversible. The gel is stiff and brittle due to the interaction of potassium ions in kappa-carrageenan gel [42]. Due to these properties, its adhesion to the gingival tissue of the oral cavity is not strong. It is easy to contaminate the wound area.

Carrageenan is a linear sulphated polysaccharide with a high molecular weight. These agents can form a gel matrix, absorbing water and solute (solute). K-carrageenan hydrogel causes effective fluid absorption, high elasticity, flexibility, soft texture, good mechanical strength, transparency, and adhesion to the wound surface by relieving pain. Hydrogel with good biocompatibility can prevent bacterial infections and control water evaporation. Synthetic materials used in this study, carbopol, gelatine, glycerol and TEA, play a role in increasing protein adsorption in hydrogel nanocomposites, which results in increased acidity and cell dispersion, increased platelet binding and reduction of blood cloth time.

To improve natural carrageenan polymers' physical and chemical properties, mixing with other natural and synthetic polymers [43]. Natural polymer materials, such as gelatin, chitosan, and sodium alginate, can increase strength, have antibacterial properties, and provide flexibility [43,44]. Polyvinyl alcohol (PVA) or polyethylene glycol (PEG) is a synthetic polymer that can increase flexibility and reduce brittleness [45-48]. Its brittle and rigid texture results in poor flexibility and suboptimal adaptability to the dynamic and moist environment of the oral cavity. This leads to lower adhesiveness, which may compromise the stability of the dressing on the wound surface and increase the risk of contamination or premature detachment. The newly formed epithelium's thickness was slightly lower than that of the commercial dressing group, indicating that cell proliferation and differentiation may not be optimal. Other studies have shown these additives to improve mechanical integrity, moisture retention, and tissue adhesion, potentially enhancing wound healing outcomes. In addition, the current study was limited to a short-term observation period of seven days. Long-term outcomes were not evaluated, including full reepithelialization, remodelling, epithelial integrity, and potential inflammatory or immune responses. Future studies should optimize the formulation of carrageenan-based hydrogels by incorporating natural or synthetic polymers to improve mechanical and adhesive properties. Moreover, extending the observation period and exploring molecular mechanisms involved in the healing response could provide a deeper understanding of carrageenan's role in tissue regeneration. Investigating the controlled release of growth factors or antimicrobial agents within the carrageenan matrix may enhance its functionality as a next-generation oral wound dressing.

Conclusion

It was concluded that wound closure using a wound dressing in the mouth cavity accelerates epithelium formation. Carrageenan-based wound dressings can increase the formation of epithelium, which has the same potential as wound dressings produced by factories.

Acknowledgements

Thank you to the research institution for providing financing support and the entire team that has collaborated in completing this research.

References

1. Kang S, Jang EJ, Jo HM, Kang SS, Lee MS, et al. (2022) Effects of a Topically Applied Oral Wound Dressing Film on Intra-oral Wound Healing in Rabbits. In Vivo 36(4): 1745-1752.

2. Soheilifar S, Bidgoli M, Faradmal J, Soheilifar S (2015) Effect of Periodontal Dressing on Wound Healing and Patient Satisfaction Following Periodontal Flap Surgery. J Dent 12(2): 151-156.

3. Kuznetsova TA, Andryukov BG, Besednova NN, Zaporozhets TS, Kalinin AV (2020) Marine algae polysaccharides as basis for wound dressings, drug delivery, and tissue engineering: A review. J Mar Sci Eng 8(7).

4. Firoozbahr M, Kingshott P, Palombo EA, Zaferanloo B (2023) Recent Advances in Using Natural Antibacterial Additives in Bioactive Wound Dressings. Pharmaceutics 15(2): 644.

5. Bandol Utomo BS, Fransiska D, Darmawan M (2016) Formulasi Hidrogel dari Polivinil Pirolidon dan k/i-Karaginan untuk Bahan Pembalut Luka. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 11(1): 55.

6. de Silva DA, Hettiarachchi BU, Nayanajith LDC, Milani MDY, Motha JTS (2011) Development of a PVP/kappa-carrageenan/PEG hydrogel dressing for wound healing applications in Sri Lanka. J Natl Sci Found 39(1): 25-33.

7. Beaumont M, Tran R, Vera G, Niedrist D, Rousset A, et al. (2021) Hydrogel-Forming Algae Polysaccharides: From Seaweed to Biomedical Applications. Biomacromolecules

22(3): 1027-1052.

8. Indahyani DE, Praharani D, Barid I, Tri A, Handayani W, et al. (2019) Aktivitas Antioksidan dan Total Polisakarida Ekstrak Rumput Laut Merah, Hijau dan Coklat dari Pantai Jangkar Situbondo (Antioxidant Activity and Total Polysaccharide of Red, Green and Brown Seaweed from Jangkar Situbondo Coastal). Stomatognatic 16(2): 64-69.

9. Tavakolian M, Munguia Lopez JG, Valiei A, Islam MS, Kinsella JM, et al. (2020) Highly Absorbent Antibacterial and Biofilm-Disrupting Hydrogels from Cellulose for Wound Dressing Applications. ACS Appl Mater Interfaces 12(36): 39991-40001.

10. Wang J (2018) Neutrophils in tissue injury and repair. Cell Tissue Res 371(3): 531-539.

11. Toma AI, Fuller JM, Willett NJ, Goudy SL (2021) Oral wound healing models and emerging regenerative therapies. Translational Research 236: 17-34.

12. Indahyani DE, Barid I, Praharani D, Probosari N (2024) Effect of Oral Wound Dressing Base on Red Seaweed Carrageenan on Gingiva Fibroblast Culture Cell Viability. Malaysian Journal of Medicine and Health Sciences 20: 186-193.

13. Struck MB, Andrutis KA, Ramirez HE, Battles AH (2011) Effect of a Short-term Fast on Ketamine–Xylazine Anesthesia in Rats. J Am Assoc Lab Anim Sci 50(3): 344.

14. Margraf A, Lowell CA, Zarbock A (2022) Neutrophils in acute inflammation: current concepts and translational implications. Blood 139(14): 2130-2144.

15. Chen L, Deng H, Cui H, Fang J, Zuo Z, et al. (2017) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9(6): 7204.

16. Toma AI, Fuller JM, Willett NJ, Goudy SL (2021) Oral wound healing models and emerging regenerative therapies. Translational Research 236(404): 17-34.

17. Nirenjen S, Narayanan J, Tamilanban T, Subramaniyan V, Chitra V, et al. (2023) Exploring the contribution of pro-inflammatory cytokines to impaired wound healing in diabetes. Front Immunol 14: 1216321.

18. Luchian I, Goriuc A, Sandu D, Covasa M (2022) The Role of Matrix Metalloproteinases (MMP-8, MMP-9, MMP-13) in Periodontal and Peri-Implant Pathological Processes. Int J Mol Sci 23(3): 1806.

19. Renò F, Pagano CA, Bignotto M, Sabbatini M (2025) Neutrophil Heterogeneity in Wound Healing. Biomedicines 13: 694.

20. Nuutila K, Eriksson E (2021) Moist Wound Healing with Commonly Available Dressings. Adv Wound Care 10(12): 685.

21. Gou Y, Hu L, Liao X, He J, Liu F (2024) Advances of antimicrobial dressings loaded with antimicrobial agents in infected wounds. Front Bioeng Biotechnol 12: 1431949.

22. Negut I, Grumezescu V, Grumezescu AM (2018) Treatment Strategies for Infected Wounds. Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry 23(9): 2392.

23. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, et al. (2014) Epithelialization in Wound Healing: A Comprehensive Review. Adv Wound Care 3(7): 445.

24. Kuninaka Y, Ishida Y, Ishigami A, Nosaka M, Matsuki J, et al. (2022) Macrophage polarity and wound age determination. Scientific Reports 12(1): 1-11.

25. Wilson SE (2021) Interleukin-1 and Transforming Growth Factor Beta: Commonly Opposing, but Sometimes Supporting, Master Regulators of the Corneal Wound Healing Response to Injury. Invest Ophthalmol Vis Sci 62(4): 8.

26. Bierbaumer L, Schwarze UY, Gruber R, Neuhaus W (2018) Cell culture models of oral mucosal barriers: A review with a focus on applications, culture conditions and barrier properties. Tissue Barriers 6(3): 1479568.

27. Tan ST, Winarto N, Dosan R, Aisyah PB (2019) The Benefits Of Occlusive Dressings In Wound Healing. Open Dermatol J 13(1): 27-33.

28. Clark RAF, Lanigan JM, DellaPelle P, Manseau E, Dvorak HF, et al. (1982) Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J Invest Dermatol 79(5): 264-269.

29. Obedencio GP, Nuccitelli R, Isseroff RR (1999) Involucrin-Positive Keratinocytes Demonstrate Decreased Migration Speed but Sustained Directional Migration in a DC Electric Field. Journal of Investigative Dermatology 113(5): 851-855.

30. Sanjivkumar M, Chandran MN, Suganya AM, Immanuel G (2020) Investigation on bio-properties and in-vivo antioxidant potential of carrageenans against alloxan induced oxidative stress in Wistar albino rats. Int J Biol Macromol 151: 650-662.

31. Chen Z, Du C, Liu S, Liu J, Yang Y, et al. (2024) Progress in biomaterials inspired by the extracellular matrix. Giant

19: 100323.

32. Neamtu B, Barbu A, Negrea MO, Berghea Neamţu C Ştefan, Popescu D, et al. (2022) Carrageenan-Based Compounds as Wound Healing Materials. Int J Mol Sci 23(16).

33. Ferraz MP (2025) Wound Dressing Materials: Bridging Material Science and Clinical Practice. Applied Sciences 15(4): 1725.

34. Ahmad N (2022) In Vitro and In Vivo Characterization Methods for Evaluation of Modern Wound Dressings. Pharmaceutics 15(1): 42.

35. Wang C, Zhang X, Fan Y, Yu S, Liu M, et al. (2024) Principles and Design of Bionic Hydrogel Adhesives for Skin Wound Treatment. Polymers 16(13): 1937.

36. Graceffa V, Zeugolis DI (2019) Carrageenan enhances chondrogenesis and osteogenesis in human bone marrow stem cell culture. Eur Cell Mater 37: 310-332.

37. Amruth P, Akshay P, Rosemol Jacob M, Joy JM, Mathew S (2024) Developmental prospects of carrageenan-based wound dressing films: Unveiling techno-functional properties and freeze-drying technology for the development of absorbent films-A review. Int J Biol Macromol 276: 133668.

38. Lu P, Ruan D, Huang M, Tian M, Zhu K, et al. (2024) Harnessing the potential of hydrogels for advanced therapeutic applications: current achievements and future directions. Signal Transduction and Targeted Therapy 9(1): 1-66.

39. Xiao Z, Li Q, Liu H, Zhao Q, Niu Y, et al. (2022) Adhesion mechanism and application progress of hydrogels. Eur Polym J 173: 111277.

40. Peng J, Zhao H, Tu C, Xu Z, Ye L, et al. (2020) In situ hydrogel dressing loaded with heparin and basic fibroblast growth factor for accelerating wound healing in rat. Materials Science and Engineering: C 116: 111169.

41. Kraithong S, Theppawong A, Lee S, Huang R (2023) Understanding of hydrocolloid functions for enhancing the physicochemical features of rice flour and noodles. Food Hydrocoll 142: 108821.

42. Rahmawati I, Liviawaty E, Intan Pratama R, Junianto (2023) View of Carrageenan in Seaweed (Eucheuma sp.) and Use of Carrageenan in Fishery Food Products: A Review. Asian J Fish Aqu Res 23(6): 1-10.

43. Kruk K, Winnicka K (2022) Alginates Combined with Natural Polymers as Valuable Drug Delivery Platforms. Mar Drugs 21(1): 11.

44. Remya S, Bindu J (2025) Chitosan and Sodium Alginate: The Potential of Natural Biopolymers of Marine Origin in Development of Biodegradable Packaging Material. Fishery Technology 62(1).

45. Mohammed MI, El-Sayed F (2023) PEG's impact as a plasticizer on the PVA polymer's structural, thermal, mechanical, optical, and dielectric characteristics. Opt Quantum Electron 55(13): 1-22.

46. Prajapati VD, Maheriya PM, Jani GK, Solanki HK (2014) Carrageenan: A natural seaweed polysaccharide and its applications. Carbohydr Polym 105(1): 97-112.

47. Zhang L, Wang Y, Yang M, Yu W, Zhao Z, et al. (2024) An Injectable, Self-Healing, Adhesive Multifunctional Hydrogel Promotes Bacteria-Infected Wound Healing. Polymers 16(10): 1316.

48. Hardman D, Thuruthel TG, Iida F (2022) Self-healing ionic gelatin/glycerol hydrogels for strain sensing applications. NPG Asia Materials 14(1): 1-13.