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Original Article

CLINICAL PARAMETERS OF CHITOSAN ORAL WOUND DRESSING FILM IN THE GINGIVAL INCISIONS OF WISTAR RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

Objective: Oral Wound Dressing Film (OWDF) prevents bacterial infections and protects newly formed tissue. The periodontal surgical treatment causes incised wounds, which may result in excessive pain. Periodontal dressings in film preparation are considered easier to apply. Chitosan is effective in accelerating wound healing because it's mucoadhesive, biocompatible, biodegradable, antimicrobial, and non-toxic properties. The research aims to analyze the effectiveness of Oral Wound Dressing Chitosan Film on clinical Landry's Healing Index and vascularization in the wound healing process after wistar rat gingival incision.

Methods: *In vivo* experimental research with a post-test control group design of 27 male wistar rats in three treatment groups with oral wound dressing film chitosan 2.5%, Ora-Aid®, and placebo. Ora-Aid®used as positive control because it's widely commercialize tomaintain the humidity in the oral environment and improve wound healing. Clinical parameters were examined with Landry's Healing Index and histological parameters of vascularization on d 3, 7, and 14. Data were analyzed using kruskal-wallis and mann-whitney.

Results: There were significant differences in vascularization between the groups on 3rd d (4.20±0.200), 7th d (5.60±0.600) and 14th d (7.20±0.872) with p value<0.05. There were differences in vascularization (p<0.05) between the two groups except for vascularization 3rd d, 7th d and 14th d between Oral Wound Dressing Film chitosan and Ora-Aid® groups (p>0.05).

Conclusion: Clinical and vascularization parameters increased after the application of an Oral Wound Dressing Film containing chitosan in the post-gingival incision wound healing process in Wistar rats (*Rattus norvegicus*).

Keywords: Oral wound dressing film, Chitosan, Wound healing

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INTRODUCTION

Opened post-surgical periodontal wounds will have the potential for complications characterized by infection, bleeding, and swelling, which can inhibit the healing process [1]. Maintaining the stability of post-surgical wounds is essential, so using a material that can act as a post-surgical wound protector is necessary [2, 3]. Periodontal dressings are a material for dressing periodontal tissues that are placed after periodontal surgery [4]. Periodontal dressings function to maintain the position of the flap, protect the newly formed tissue, prevent bacterial infection, minimize the occurrence of post-surgical pain, provide additional support to the soft tissue, and protect the surgical area from mechanical trauma when eating and drinking, but have the disadvantage of not accelerating wound healing [3–7].

Several efforts were made to improve the quality of dressing materials, such as introducing antibacterial chlorhexidine or antibiotics, enhancing the healing process, and preventing infection [8]. Other possible problems are the emergence of bacterial resistance when antibiotics are involved, the risk of sensitisation and allergy, and the growth of candidiasis. Alternative active compounds should be identified as another option to prevent the adverse effects of antibiotics in the dressing. It is more useful if the dressing material does not induce microbial plaque retention and has no impact on the masticatory process [8].

One of the materials that has recently been developed and has an essential position as a natural resource biomaterial is chitosan. Chitosan has various properties and characteristics, including biodegradable, biocompatible, non-toxic, hydrophilic, anti-bacterial, and affects the wound healing [9, 10]. Chitosan contributes to tissue healing, so it has been widely used as a periodontal option for wound healing of the oral tissue. Chitosan has hemostatic and antimicrobial activities that play a crucial function in wound healing [10]. Chitosan has antibacterial effects against periodontal pathogenic bacteria [11]. Chitosan can recruit and activate neutrophils and macrophages and stimulate the angiogenesis process so as to accelerate the healing process [10].

Wound healing comprises four phases: hemostasis, inflammation, proliferation, and remodeling. An incised wound causes a biological response in the form of wound healing [11]. After the inflammatory phase, there will be a proliferation phase, which is characterized by the formation of new blood vessels. Angiogenesis, or the formation of new blood vessels, is critical to the wound-healing process. This process supplies oxygen, nutrients, and inflammatory cells and eliminates tissue experiencing necrosis [11]. Pereira, in 2020, conducted research using 1.5% chitosan as a wound dressing material after skin excision surgical wounds. The study found that wounds treated with chitosan had more collagen deposition, re-epithelialization, and neovascularization, as demonstrated by enhanced keratinocyte proliferation, vascular branching, and blood vessel growth [12].

Various types of periodontal dressings have been identified by research regarding the benefits of using dressings [4]. Various dressing materials, mucoadhesive dressings with the commercial name Ora-Aid® (TBM Co., Gwangju, Korea) are starting to be widely used. The Ora-Aid® has two layers: a lipophilic outer layer that prevents the inner layer from dissolving quickly, and a hydrophilic inner layer that reacts with the oral cavity's mucosa to form a gel, absorbing wound exudate and micro bleeding. After applying the Ora-Aid® to a fresh socket, the inner layer reacted with the oral mucosa and the outside layer dissolved within six to eight hours [13].

Clinical research by Min H. *et al.* in 2020 to evaluate healing and patient satisfaction with the use of OWDF was carried out on 24 patients after curettage surgery. The data state that all patients reported less pain, bleeding, and discomfort after surgery compared

to the side that did not use OWDF. Oral Wound Dressing film can provide comfort and less pain [14]. The study aims were designed to analyze the effectiveness of Oral Wound Dressing Chitosan Film on the clinical Landry's Healing Index and vascularization histological in the wound healing process after wistar rat gingival incision.

MATERIALS AND METHODS

This *in vivo* laboratory trial uses a post-test control group design to examine clinical and histological data. The Research Ethics Committee accepted this study under the registration number 0785/KEPH-FMIPA/2023. The samples utilized in this investigation were male wistar rats (*Rattus novergicus*) aged 2-3 mo, weighing 180-200 g and in good health, with up to 27 rats distributed evenly into three groups. The 27 male wistar rats (*Rattus novergicus*) were placed into three treatment groups (9 rats/each) due to adequacy and ethical considerations. Group 1 received oral wound dressing film with chitosan; Group 2 received oral wound dressing film ora-Aid® (TBM Corp., Canada); and Group 3 received oral wound dressing film with no active ingredients (placebo).

The oral wound dressing film containing chitosan was manufactured by dissolving 250 mg chitosan powder (molecular weight 240-300 kDa and 70% degree of deacetylation) in 3% acetic acid poured into a glass beaker and mixed for 24 h using a magnetic stirrer at room temperature. After homogeneous, the pH of chitosan was checked. 50 mg PVP was dissolved into distilled water and then covered with aluminum foil for 24 h. Then, CMC-Na was dissolved with hot water for 20 min. Chitosan, PVP, and CMC-Na polymers were combined by adding 15 ml of polysorbate to combine the three polymers. The three polymers were set in a petri dish at room temperature (20 °C-25 °C) for 30 min to remove trapped air bubbles. The petri dish was dried in an oven at 40 °C-42 °C for 2-3 d to obtain a film-shaped preparation. After the preparation was dry, the film was cut to a size of 5 mm x 2 mm using a blade [15]. After the preparation was made, weight uniformity, pH test, folding resistance and disintegration time were carried out.

The incision of the rats was carried out by intraperitoneal injection anesthesia of ketamine and xylazine mixture with 5 mg/kg of body weight dose. An incision was made on the labial gingival surface area of the mandibular central incisor of rats with an incision length of 3 mm until it touched the bone in one pull using a sterile stainless steel no 15 °C scalpel that was changed every three incisions. Bleeding was adequately controlled, and Oral Wound Dressing Film measuring 5 mm x 2 mm was applied to the surgical area to cover the entire incision with interrupted suture. Chitosan Oral Wound Dressing Film was applied to Group 1, Ora-Aid® Oral Wound Dressing Film was used to Group 2, and oral wound dressing film without active ingredients (placebo) was applied to Group 3. For seven days, all groups received oral wound dressing film twice daily, in the morning and evening.

The Clinical parameters were assessed using Landry's Healing Index [8]. Landry's healing index was performed by visually inspecting and palpating the surgical incision area of each group with the aid of a loupe and headlamp for clearer vision, then assessing tissue color, bleeding response to palpation, presence of granulation tissue, incision edge characteristics, and incidence of suppuration on d 3, 7, and 14. This index assesses clinical wound healing using a score of 1 to 5, with very poorly healed wounds scoring one and best healing wounds scoring 5.

The rats were sacrificed for the preparation procedure of histological examination. Rats were euthanised using the neck dislocation technique. The neck was separated from the head using a scalpel and blade. Rat mandibular segments were taken from the head using a blade and surgical scissors to obtain intact mandibular pieces. The obtained mandibular segment was washed with saline solution to clean the sample from blood and bacterial contamination. The mandibular segments were placed in a container containing 10% Neutral Buffer Formalin to maintain the sample's integrity for 24 h. The rest of the rat's body parts were buried following animal ethics. The procedure for making histological preparations of mandibular segments was carried out by decalcification with 2% ethylenediaminetetraacetic acid (EDTA), 10% buffered formalin fixation, liquid paraffin infiltration, embedding, sectioning, and staining using hematoxylin dye on randomly region of mandibular segments.

On d 3, 7, and 14, histological preparations were observed using a binocular microscope at 400x magnification with the help of Image Processing Software. Assessment was obtained by measuring the thickness of the epithelium, counting the density of collagen fibers, counting the number of vascularisations in each visual field (average lumen area), and calculating the average number for each of the five visual field areas.

The data obtained from the study were processed using a computerized system using Statistical Package for the Social Sciences (SPSS) V.29 software (IBM SPSS Statistics; IBM Corp; f861ca2610c844047962). Data were analyzed using univariate analysis to determine the mean and standard in all groups on d 3, 7, and 14. Data were analyzed using bivariate analysis through the normality test using the Shapiro-Wilk and the homogeneity test using the Levene Test. Then, Kruskal-Wallis and Mann-Whitney tests were conducted because the data were not normally distributed and homogeneous.

RESULTS

Characteristics examination of oral wound dressing chitosan film and placebo

Weight uniformity test

Testing the uniformity of chitosan and placebo weights by weighing Oral Wound Dressing measuring 3 cm x 1 cm using analytical scales was performed 5 times [18].

pH test

The pH test was carried out by cutting the Oral Wound Dressing Film chitosan and placebo measuring 3 cm x 1 cm, then putting it into 100 ml aquabidest till at room temperature. The pH measurement is carried out when the film has dissolved using a digital pH meter on the oral wound dressing film. The results of the pH measurement were recorded and carried out five times [19, 20].

Folding durability test

The fold resistance test is performed by repeatedly folding one film in the exact location until it breaks or folds [17, 20].

Time crush test

The disintegration time test was carried out with the preparation *of Oral Wound Dressing Film,* chitosan and placebo. Data of disintegration time test is calculated using a disintegration tester. The film that has been cut to size is inserted into the tool hole and then operated using aquabidestillate as a solvent medium. The disintegration time measurement tool remains operational until the time the film tears, disintegrates, or dissolves [17].

Clinical parameters of gingival post-incision wound

Based on table 2, Landry's healing index in the gingival post-incision wound on day 3, the majority of all groups showed a poor score, namely 50% of the gingiva was red, the response to palpation showed bleeding, and the incision margin was not epithelialized with open connective tissue but no suppuration (fig. 2). Landry's healing index on day 7, all samples in the Oral Wound Dressing Film chitosan group showed a good index. In the Ora-Aid® group, there were samples with good and very good scores, while placebo had good and very poor ones (fig. 3). Landry's healing index on day 14, samples in the chitosan oral wound dressing film and placebo groups showed excellent and good scores, while those in the Ora-Aid® Oral Wound Dressing Film group all showed excellent scores (fig. 4) In distinguishing "very good" from 'excellent' is viewed through the color of the tissue, which is<25% red "very good" and all pink tissue color indicates "excellent".

Table 1: The film weight, thickness, pH, folding durability, and time crush examination of oral wound dressing chitosan film, chitosan and placebo

Weight uniformity test					
Formulation	**Weight (g)**	**Weight (g)**	**Weight (g)**	**Weight (g)**	**Weight (g)**
Chitosan	0.2721	0.2558	0.230	0.234	0.223
Placebo	0.1977	0.223	0.221	0.202	0.221
pH test					
Formulation	pH Test	pH Test	pH Test	pH Test	pH Test
Chitosan	5.96	5.96	5.95	5.91	5.95
Placebo	5.94	5.94	5.95	5.95	5.96
Folding durability test					
Formulation	Folding durability test (repetition)	Folding durability test (repetition)	Folding durability test (repetition)	Folding durability test (repetition)	Folding durability test (repetition)
Chitosan	10	10	10	10	10
Placebo	9	9	9	9	9
Time crush test					
Formulation	Destruction time (min)	Destruction time (min)	Destruction time (min)	Destruction time (min)	Destruction time (min)
Chitosan	210	225	210	225	225
Placebo	8	8	8	8	8

Table 2: Evaluation results of landry healing index of post-incision wound on gingiva

Period	**Group**	**Landry's healing index n (mean ± SD)**				
		Very poor	**Poor**	**Good**	**Very good**	**Excellent**
Day 3	Chitosan	0	2 (66.7%)	0	1 (33.3%)	0
	Ora-Aid®	0	2 (66.7%)	0	1 (33.3%)	0
	Placebo	0	2 (66.7%)	0	1 (33.3%)	0
Day 7	Chitosan	0	0	0	3 (100%)	0
	Ora-Aid®	0	0	0	2 (66.7%)	1 (33.3%)
	Placebo	1 (33.3%)	0	0	2 (66.7%)	0
Day 14	Chitosan	0	0	0	1 (33.3%)	2 (66.7%)
	Ora-Aid®	0	0	0	0	3 (100%)
	Placebo	0	0	0	2 (66.7%)	1 (33.3%)

SD= Standart deviation

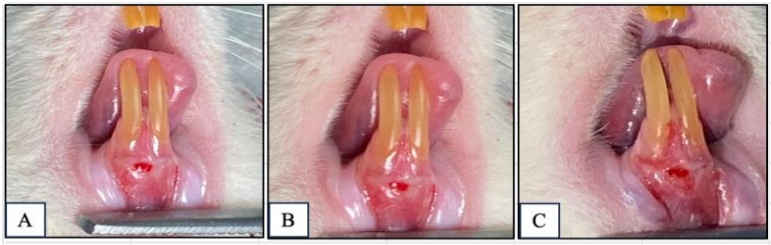

Fig. 1: Baseline clinical aspect of wound healing after rat gingival incision and application of OWDF (A) Chitosan (B) Ora-Aid® (C) Placebo

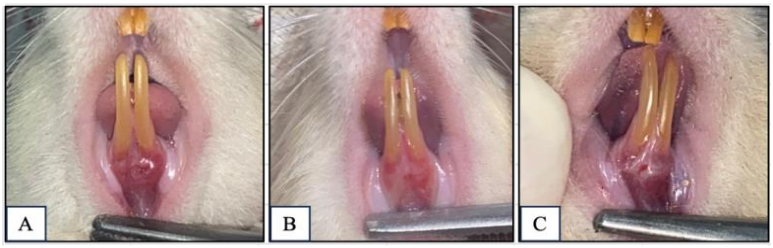

Fig. 2: Third-day clinical aspect of wound healing after rat gingival incision and application of OWDF (A) Chitosan (B) Ora-Aid® (C) Placebo

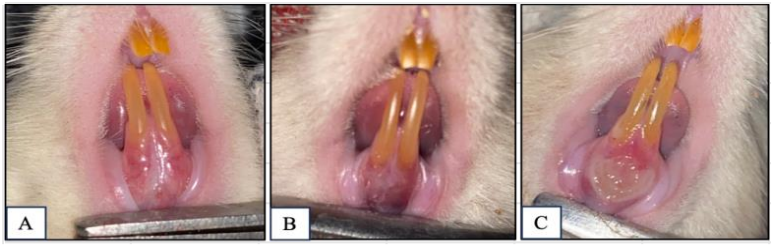

Fig. 3: One week clinical aspect of wound healing after rat gingival incision and application of OWDF (A) Chitosan (B) Ora-Aid® (C) Placebo

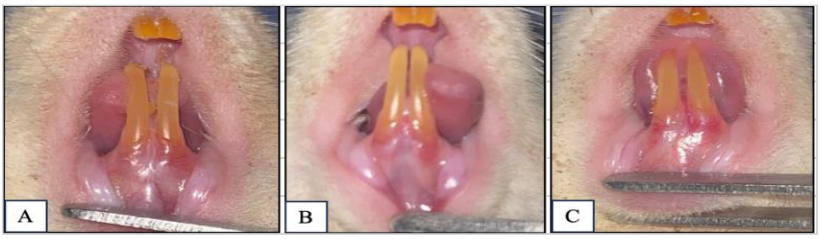

Fig. 4: Two-week clinical aspect of wound healing after rat gingival incision and application of OWDF (A) Chitosan (B) Ora-Aid® (C) placebo vascularisation in gingival post-incision wounds

Kruskal-Wallis statistical test to assess differences in vascularization across groups on d 3, 7, and 14 and Post Hoc LSD test to assess differences in vascularization of each group.

The number of vascularizations formed was more significant in the Ora-Aid®group, followed by the chitosan group and the placebo group on d 3, 7, and 14, and this difference was statistically significant ($p<0.05$). The results of the Kruskal-Wallis statistical test showed that there were substantial differences in vascularization between the Oral Wound Dressing Film chitosan, Ora-Aid®, and placebo groups on d 3, 7, and 14 ($p<0.05$).

Table 4 presents significant differences in vascularization after the application of Oral Wound Dressing Film chitosan, Ora-Aid®, and placebo in wound healing post-gingival incision of wistar rats on d 3, 7, and 14 between 2 different treatment groups.

Based on table 4, the results of the Mann-Whitney test show that there is a significant difference in vascularization between the two groups in all treatment groups on the 3rd, 7th, and 14th d after application of oral wound dressing film ($p<0.05$), except in the oral wound dressing film treatment group chitosan and Ora-Aid® d 3, 7 and 14 which showed no significant results ($p>0.05$).

Table 3: Results of data statistical test on differences in vascularization after gingival incision in wistar rats

Significant difference test					
Average vascularisation ($\bar{x}$±SD)					
Time	Group				*p-value*
	Chitosan	Ora-Aid®		Placebo	
Day 3	4.20±0.200	5.00±1.039		1.67±0.642	0.035*
Day 7	5.60±0.600	6.47±0.306		2.27±0.611	0.032*
Day 14	7.20±0.872	8.00±0.721		2.67±0.611	0.049*
* Kruskal-Wallis p-value<0.050 = significant between groups					
SD= Standart Deviation					
mean difference test					
Group		Comparison	*p-value*		
			Day 3	Day 7	Day 14
Chitosan		Ora-Aid®	0.105	0.077	0.275
Chitosan		Placebo	0.050*	0.050*	0.050*
Ora-Aid®		Placebo	0.046*	0.050*	0.050*
*Mann-Whitney p-value<0.05 = significant between 2 groups					
SD= Standart Deviation					

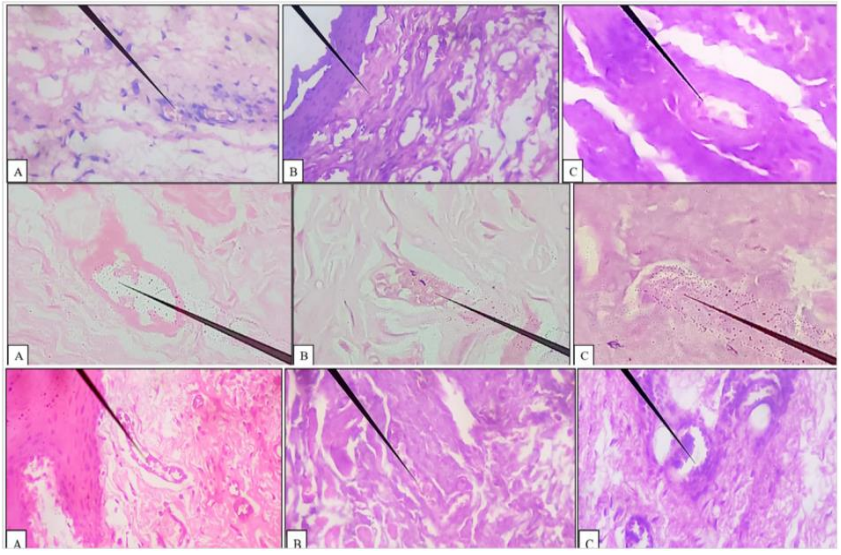

Fig. 5: Histological vascularization on day 14 after application of oral wound dressing film per average lumen area (A) Chitosan (B) Ora-Aid® (C) Placebo. The amount of vascularization is calculated histologically by counting the number of newly formed blood vessels indicated by the microscope's pointer.

DISCUSSION

The results of descriptive analysis of wound healing in mice after clinical incision showed that administration of OWDF containing chitosan provided a better wound healing effect compared to the administration of placebo as a negative control. However, this healing effect was no better than the positive control using OWDF Ora-Aid®. This can be seen on the 7th day of observation, namely that the mice given Ora-Aid®incision margins have begun to re-pitalize with<25% of the gingiva being red when compared to mice given OWDF chitosan, where ≥25% to<50% of the gingiva is red. Meanwhile, in the group given a placebo, the incision margins had not yet been capitalized, there was still bleeding, and ≥50% of the gingiva was red. This can happen because chitosan has the unique ability as a hemostatic agent, which can replace the role of platelets in blood clotting so that it can function as a wound-covering membrane during the healing process [21]. This ability is based on the contact of chitosan with blood cells on the outer membrane of erythrocytes, and platelets are drawn to the potentially reactive amino and hydroxyl groups of chitosan, resulting in platelet activation and the development of thrombus. It can hasten blood coagulation.

The application of Oral Wound Dressing Film containing chitosan effectively accelerates wound healing after gingival incision compared to the placebo group. This may occur because chitosan has a unique ability as a hemostatic agent that can replace the role of platelets in blood clotting so that it can function as a wound closure membrane during the healing process [21]. This capacity is dependent on how chitosan reacts with blood cells. The polarized outer membrane of erythrocytes and platelets will be attracted to the positively charged reactive amino and hydroxyl groups of chitosan, causing platelet activation and thrombus formation [21, 22]. Chitosan hydrogels adhere firmly to the wound and cover the wound during healing. Chitosan contributes to better granulation tissue formation and epithelialization in wounds treated with chitosan hydrogel [23]. This is in line with the research of Putra *et al.* stated that the administration of chitosan could accelerate incisional wound healing as evidenced by reduced redness and relatively rapid wound closure [21].

Chitosan can enhance the wound healing process due to its active compound of N-acetyl glucosamine group [9, 24]. This compound can accelerate PMN migration and induce macrophages by stimulating the macrophage chemotaxis process, which then releases PDGF and TGF-β. N-acetyl glucosamine monomers can tie to mannose receptors on macrophages [25]. The mannose receptors on macrophages and hepatic endothelial cells filter out lysosomal enzymes, glycopeptide fragments from collagen, and pathogenic bacteria, minimizing tissue damage following injury. This receptor binds to mannose, fucose, and N-acetylglucosamine (GlcNAc) residues on the targets. The mannose receptor (CD206) is expressed on macrophages, dendritic cells, and hepatic sinusoidal endothelial cells [25].

Chitosan is internalized by macrophages, triggering cell migration and proliferation [26]. Macrophage cell activation increases metabolic activity, growth factor secretion, and angiotensin, which triggers the formation of new blood vessels and improves blood supply distribution and cell regeneration processes, resulting in faster wound healing [21, 23]. In wound recovery, redness occurs due to the growth of new capillaries in the wound area [22]. In the chitosan Oral Wound Dressing Film treatment group, post-incision gingival redness was seen in more than 50% of the gingival area on day 3, reduced to 25% on day 7 and day 14, and the gingiva was completely pink. This new blood vessel formation accelerates cell regeneration and tissue normalization. Neocapillaries are formed through the mitotic activity of vascular endothelial cells, which then migrate to the wound area [1]. The main function of neocapillary formation is to provide vitamins, minerals, glucose, and amino acids to fibroblasts, which enhance collagen formation and rid the tissue of necrosis, foreign bodies, and infection, thereby accelerating the wound healing process [24]. The faster capillary formation will accelerate wound healing by increasing blood supply [12]. Blood supply is essential to meet the active metabolic needs of cells, which in turn accelerates tissue regeneration [25]. Capillaries in fresh granulation tissue are essential as developing cells require adequate energy and nutrients from the blood [25]. In the placebo group, bleeding was clinically evident on day 3, while less bleeding was observed in the chitosan OWDF group. This is because chitosan has a distinctive ability as a hemostatic agent that can replace the role of platelets in blood clotting, thus serving as a wound closure film during the healing process [27-29].

Because of its multifunctional biological features, chitosan has shown great promise as a therapeutic agent for promoting periodontal wound healing. Its natural antibacterial activity, derived from positively charged amino groups that destroy bacterial cell walls, renders it efficient against periodontal infections, including Porphyromonas gingivalis and Streptococcus mutans [30]. Chitosan reduces pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6, leading to a more favorable healing environment [31]. Chitosan also promotes fibroblast proliferation, collagen synthesis, and osteoblastic activity, which aids in tissue regeneration and accelerates wound healing [32]. Furthermore, its hemostatic qualities promote clot formation and release of growth factors like PDGF and TGF-β1, which enhance the healing process.

In this study, the placebo group developed abscesses. This condition occurs due to bacterial infection since a placebo has no antibacterial effect [33]. The abscessed rat group looked weak and could not eat due to pain and swelling. This happens because the placebo does not contain active ingredients to protect the wound from contamination during the healing process [33]. This is consistent with the study by Loekito *et al.* in 2018, which states that chitosan has antibacterial power against bacterial biofilms. This study also supports the theory that chitosan is an antibacterial ingredient [34].

Chitosan contains N-acetylglucosamine, which can bind to FGF (Fibroblast Growth Factor) and stimulate angiogenesis to accelerate the healing process [20]. The results of a study conducted by de Jesus *et al.* stated that the effect of 3% chitosan hydrogel in the healing process of the oral mucosa after tooth extraction showed that 3% chitosan hydrogel could accelerate wound healing with fewer complications compared to without chitosan [10]. In the wound healing process, many blood vessels are needed to assist the healing process by increasing oxygen circulation and tissue perfusion [35]. Blood vessels generated from the angiogenesis process play a role in maintaining the continuity of function of various affected tissues; besides that, they can provide oxygen supply, nutrients, and inflammatory cells and are able to eliminate tissue affected by necrosis. The greater the number of blood vessels found, the faster the tissue recovery [36].

The application of chitosan can expedite wound healing. Sularsih *et al.* stated that using 1% chitosan gel can release TNF-α cytokine to activate macrophage cells on day 3 to accelerate the healing process [37]. Another study by Aditya *et al.* revealed that using 3% chitosan gel could improve the number of macrophage cells, which helps accelerate wound healing [38]. Chitosan promotes wound healing by stimulating and regulating inflammatory cells such as neutrophils, macrophages, fibroblasts, and endothelial cells, as well as increasing the creation of granulation fiber [39]. Activation of macrophage cells will increase metabolic activity in the form of secretion of growth factors metabolic activity in the form of secretion of growth factors and angiopeptins that stimulate the formation of new blood vessels to increase the distribution of blood supply and the process of cell regeneration so that wound healing occurs faster [40]. Developing new blood vessels or angiogenesis is a phase stimulated by high energy requirements for cell proliferation. In addition, angiogenesis is also required to regulate vascularisation damaged by the wound and stimulated by high lactate conditions, acidic pH levels, and decreased oxygen tension in the tissue [40-42].

The considerable difference in disintegration times—210 to 225 min for the chitosan-based oral wound healing film versus only 8 min for the placebo—demonstrates fundamental changes in formulation structure and composition. One important factor is the crosslink density of the chitosan network, which generates a strong hydrogen-bonded structure that provides mechanical stability and resistance to disintegration in aqueous conditions [43]. The addition of formulation additives such as polyvinylpyrrolidone (PVP) and sodium carboxymethylcellulose (CMC-Na), which contribute to the film's viscosity and stability, may also explain the prolonged

disintegration period [44]. PVP strengthens the film, extending its disintegration period, whilst CMC-Na works as a gelling agent, reducing water penetration and film dissolution. In contrast, the placebo, which lacks these components, disintegrates quickly due to its looser and more fragile matrix [45]. These findings highlight the importance of polymer interactions and excipient selection in modifying disintegration rates, and hence the film's usefulness as an oral wound dressing.

Based on the results, it was found that OWDF chitosan is a good alternative to placebo. However, the effectiveness of OWDF chitosan cannot surpass Ora-Aid®. Some of the shortcomings of chitosan OWDF are that the duration of adhesion of chitosan OWDF is still relatively insufficient because it only lasts less than 6 h and must be assisted by using saliva. The movement of the film or swallowing by the rat sample is a factor that researchers cannot control, so some wound areas cannot be fully covered. The Ora-Aid®-treated group did not experience infection because it contains tocopherol acetate (vitamin E), carbomer, and povidone, which act as hemostatic. Ora-Aid® has optimal adhesion for 6-12 h, so wound repair becomes more significant because it is protected from bacterial irritation and physical stimuli [22].

The Ora-Aid® control outperforms chitosan in periodontal wound healing due to its antioxidant components, such as tocopherol, which significantly reduce oxidative stress and inflammation at the wound site, thereby enhancing tissue regeneration [46]. Furthermore, its superior adhesive characteristics are believed to improve retention at the wound interface, resulting in more stable healing conditions. In contrast, the efficacy of chitosan is heavily dependent on its physicochemical properties. The chitosan utilized in this investigation, with a molecular weight of 50-70 kDa and a degree of deacetylation (DDA) of roughly 75%, may not provide the ideal balance of bioadhesion and biodegradability as reported in previous studies [47].

The effect of Ora-Aid®because it contains Hepatocyte Growth Factor (HGF). HGF is a cytokine that is predominantly engaged in numerous cellular processes such as wound repair and tissue regeneration, and it is activated by a variety of host stimuli such as bacteria and inflammatory cytokines. HGF is hypothesized to aid wound healing through the mesenchymal epithelial interactions [48, 49].

The findings from this animal model study demonstrate the potential translational value of chitosan-based oral wound dressing films in human periodontal therapy. Given chitosan's biocompatibility, antimicrobial activity, and ability to promote vascularization and tissue regeneration, its application in post-surgical gingival management may improve clinical outcomes by accelerating healing, reducing infection risk, and enhancing patient comfort. These results support further investigation through human clinical trials to validate efficacy and safety in dental practice."

This study has various limitations that must be noted. The limited sample size of three animals per time point (9 rats per group) reduces statistical power and may lower the findings' dependability [50]. Furthermore, the 14 d observation period may fail to capture long-term healing outcomes such as scarring or graft reabsorption, affecting the overall treatment evaluation [51]. Mechanical properties such as tensile strength and adhesion duration were also not assessed, despite the fact that these factors are critical for understanding how the dressing would perform in real-world conditions, particularly in the oral cavity, where there is constant movement and mechanical forces [51].

CONCLUSION

Oral Wound Dressing Film chitosan can increase vascularity after gingival incision compared to the group given placebo and is effective in accelerating wound healing.

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AUTHORS CONTRIBUTIONS

Irma Ervina-Conception, writing original draft preparation and revision manuscript, data design and analysis, performed the experiments; Aini Hariyani Nasution-Supervision and visualization, revision of manuscript; Hesty Nurcahyanti-Revision manuscript, data design and performed the experiments; Harry Agusnar-Supervision and visualization, revision of manuscript; Denny Satria-Visualization and revision of manuscript.

CONFLICT OF INTERESTS

The authors state that there are no conflicts of interest in publishing this research.

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