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The Effect of White Shrimp Head Chitosan Gel (*Litopenaeus vannamei*) on Inhibitory Strength of Periodontopathogenic Bacteria and Accelerating Wound Healing (In Vitro, Histological, and Clinical Tests)

Asdar Gani[1], Nurlindah Hamrun[2], A. Mardiana Adam[1], Ermina Pakki[3], Harun Achmad[4*], M Husni Cangara[5], Husnul Khatimah Maulidina[6], Rezqy Maelani[6], Dewiayu Dewang[6]

[1]Department of Periodontology
[2]Departementof Oral Biology
[3]Department of Pharmacy
[*4]Department of Pediatric Dentistry, Dentistry Faculty of Hasanuddin University, Makassar, Indonesia
[5]Department of Anatomical Pathology
[6]Dental Student, Faculty of Dentistry, Hasanuddin University, Makassar Indonesia
Correspondence Author E-mail: harunachmader@gmail.com

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ABSTRACT

Background: Chitosan has biocompatibility, non-toxic, biodegrade ability, and polyelectrolyte properties. It has been shown to be antimicrobial and accelerate wound healing. Chitosan gel from the waste of white shrimp head can be a new innovation which is effective to inhibit periodonto-pathogenic bacteria and to accelerate wound healing. **Aim:** To determine the potential of chitosan gel from white shrimp head (Litopenaeus vannamei) waste to inhibit periodonto-pathogenic bacteria and to accelerate wound healing in white mice (Mus musculus).
Method: The research was experimental laboratory research. The design of this study was post-test only with control group design. Research on bacterial inhibition was done by 6-team, 5 times repetitions, and 5 treatments;1%, 2%, 3% chitosan gel positive control (Metronidazole disk) and negative control. The subjects of this wound healing research were 24 white mice (Mus musculus) with incisions on the back. They was divided into treatment groups and control groups with glycerol. Clinical and histological observations were carried out on days 1, 3, and 7.
Result: The Mann Whitney test results showed a significant difference in inhibition of 1%, 2%, 3% chitosan gel against the Aggregatibacter actinomycetemcomitans bacteria ($p < 0.05$). The results of the Shapiro-Wilk test showed significant differences in wound healing and the number of PMN cells between the treatment group and the control group ($p < 0.05$). Meanwhile, the number of fibroblast cells did not show a significant difference between the two groups ($p > 0.05$).
Conclusion: Chitosan gel from the white shrimp head (Litopenaeus vannamei) waste can inhibit the growth of Aggregatibacter actinomycetemcomitans bacteria and accelerate the healing of back wounds of the white mice (*Mus musculus*) with a decrease in the number of PMN cells and the increase of fibroblast t cells.
Keywords: Chitosan Gel, *Litopenaeus vannamei*, Antimicrobial, *Aggregatibacter actinomycetemcomitans*
Correspondence:
Muhammad Harun Achmad
Department of Pediatric Dentistry
Dentistry Faculty of Hasanuddin University
Makassar, Indonesia
E-mail: harunachmader@gmail.com
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INTRODUCTION

Chitosan (C6H11NO4) is a compound in the form of amorphous white with yellowish- white color, polyelectrolyte, and generally soluble in organic acids. Solubility is influenced by molecular weight and degree of deacetylation [1]

Chitin was isolated from shrimp waste through two stages namely demineralization and deproteinization. The chitin obtained was synthesized into chitosan by changing the acetamide group (-NHCOCH3) in chitin to amine group (-NH2) [2]. The reaction of the removal of the acetyl group in chitin is called the transformation of chitin into chitosan and it is used a strong base with high concentration [3].

Chitosan as a biopolymer was studied as an antimicrobial agent against various target microorganisms such as algae, bacteria, and fungi, both in vivo and in vitro in various dosage forms [4,5]. This biopolymer shows strong activity in reducing plaque and is proven as an antimicrobial in vitro against pathogenic bacteria involved in plaque formation and periodontal diseases such as Aggregatibacter actinomycetemcomitans, P. Gingivalis, and Streptococcus mutans [5]. The potential use of chitosan as an antibacterial is based on the initial interaction between chitosan and electrostatic bacteria. Chitosan has a functional group of amines (–NH2) that are positively charged very strongly to bind with the bacterial cell wall which is relatively negatively charged [6,7]

In the field of medicine today, chitosan has been used as a biomaterial to accelerate wound healing because it has beneficial capabilities as hemostatic, non-toxicity, biocompatibility, and biodegradability [8]

MATERIALS AND METHODS

Type of Research and Research Design

This research is a type of laboratory experimental research. The design of this study is post- test only with control group design. To see the inhibition of bacteria, a paper disk was used and placed on the culture medium. This method is often used to observe diameter of specific inhibitory zones of an extract. The treatment was repeated 6 times with 1%, 2%, and 3 % chitosan gel treatments. The positive control was used metronidazole disk and negative control was used glycerol. While the method for observing the application of chitosan gel to wound healing in the backs of white rats (Mus musculus) was divided into two treatment groups: the chitosan gel treatment group as a positive control and the glycerol treatment group as a negative control.

Place and time of research

This research was conducted at the Phytochemical Laboratory and Pharmaceutics Laboratory, faculty of Pharmacy, Hasanuddin University. The bacterial inhibition procedures were done in the Laboratory of Microbiology, faculty of Medicine, Hasanuddin University, Makassar. The wound procedure was performed at the Pharmacy laboratory of Pancasakti University, Makassar. Finally, the histological test procedure was done at the Anatomical Phatology laboratory, faculty of Medicine, Hasanuddin university. The study was conducted between May and October 2018.

TOOLS AND MATERIALS

The research tools used were blenders, scales, tweezers, petri dishes, bunsen, measuring cups, erlenmeyer flasks, chemical cups, test tubes, test tube racks, calipers / calipers, incubators, autoclaves, stirring rods, ovens, small tubes (containers storage), binocular microscopes, mouse cages, glass plates, sample storage containers , blades, and scalpels . The materials used in this study were, white shrimp head waste (Litopenaeusvannamei), bacterial culture Aggregatibacter actinomycetemcomitans, MHA medium (Muller Hinton Agar), white mice (Mus musculus), spiritus, aquades, aluminum foil, paper disks, metronidazole disk , filter paper, pH paper, label paper, 1.5 M HCL, 4% NaOH, 60% NaOH, 1% CH3COOH, glycerol 50%, Ketamine Anastesicum, Hematoxylin eosin, formalin, and white rat food.

Material Test Procedure

1. Raw materials

The raw material used in this study was white shrimp head waste (Litopenaeus vannamei). Shrimp head waste was collected, the shrimp scalp was then separated, and washed with water until clean. It was dried at 110-120º C temperature for ± 1 hour in oven [9]. Then, mashed using a blender. The shrimp head waste (Litopenaeus vannamei) used was 200 gr.

2. Isolation of chitin from shrimp head flour

a. Demineralization

Shrimp head powder that had been mashed was added with 1.5 M HCl solution. Shrimp head powder and 1.5 M HCl solution were mixed in a beaker with a ratio of 1:15 (w / v), then heated at 60-700ºC for 4 hours while stirring. After that, it was filtered and rinsed with distilled water to a neutral pH10.

b. Deproteination

Shrimp head powder resulting from demineralization was added with 4% NaOH solution. The mixture was put into a beaker in a1:10 ratios (w / v) [11], then heated at 60- 700 ºC temperature for 4 hours while stirring. Then filtered and rinsed with distilled water to a neutral pH[10].

3. Chitin transformation into chitosan

Chitosan synthesis was obtained from the deacetylation process using the Knorr method. The results obtained from the deproteination process (chitin) were followed by deacetylation, adding 60% NaOH in a ratio of 1:20 (w / v), then heated at a 100 -110º C temperature for 4 hours then filtered and washed with distilled water to a neutral pH10. The solid was then dried in an oven at 65º, then cooled. After that, the chitosan obtained would be characterized by solubility testing using acetic acid 12. For the solubility test 1% CH3COOH solution was used.

4. Procedure for Making Chitosan Gel

The weighed chitosan powder was put into a measuring cup and then dissolved with 1% acetic acid solution (CH3COOH). Chitosan gel was made by concentrations of 1%, 2%, 3%, and 4%. Glycerol 50% was used to get good gel consistency.

RESEARCH PROCEDURE

1. Inhibition Test

All materials were tested and bacteria Aggregatibacteria actinomycetemcomitans previously been cultured on MHA medium (Muller Hinton Agar) was prepared. Then, the paper disk was dipped in 1%, 2%, and 3% chitosan gel along with positive control (metronidazole disk) and negative control. The dipped disk was inserted into a petri dish containing a bacterial culture. Then, it was incubated with the anaerobic atmosphere at a temperature of 37ºC for 1x24 hours. Zones of inhibition were formed in each petri dish with calipers, then compared.

2. Back Streaking and Application of Chitosan Gel in Experimental Animals

Twenty four white rats were divided into 6 groups: 3 control groups and 3 experimental groups. Sample grouping technique was employing simple random sampling. Each rat was anesthetized using ketamine. The ketamine dose used was 24 ml / g BW. Chitosan gel was apllied in wounds of the treatment group, while 50% glycerol was applied in the control group. There were 8 white rats decapitated on day 1, day 3 and day 7. After the wound healing, the length was measured using a caliper first, then tissue was taken.

3. Making Tissue Preparations

Histological tissue was made as histological preparations and stained with hematoxylin- eosin to observe the number of polymorphonuclear cells (PMN) and fibroblasts using a binocular microscope with 400x magnification.

Data analysis

Data obtained from this study were collected based on measurements of bacterial inhibition zones after treatment administration. Data was then evaluated statistically. Further, parametric analysis was performed with the *Mann Whitney* Test to determine the comparison of the effectiveness of chitosan gel derived from white shrimp head waste (Litopenaeus vannamei) to inhibit *Aggregatibacter actinomycetemcomitans*.

The data obtained from the observation of the number of PMN cells and fibroblasts t, as well as the measurement of wound length with numerical data with a ratio scale, then the average number of PMN cells and fibroblasts was

calculated, including the length of the wound based on groups. The validity test of the data between two observers used the Pearson Correlation test. After the data was valid, the normality test was done first by using the Saphiro- Wilk test (N <50) and homogeneity was tested by the Levene test. This study used a one-way ANOVA test to determine the effect of 4% chitosan gel treatment on white rat wounds on the number of PMN cells and fibroblasts, as well as the wound healing length of white rats.

RESEARCH FINDING

1. Chitosan Gel Inhibitory test results for Agregatibacter actinomycetemcomitans Bacteria

The inhibition test of chitosan from waste scalp white shrimp (Litopenaeus vannamei) against bacteria Aggregatibacter actinomycetemcomitans showed that Aggregatibacter actinomicetemcomitans on chitosan gel 1%, 2%, and 3% were great against bacteria compared with the positive control (Metronidazole disk) and negative control

Figure 1: Inhibition zone results after 1x24 hour incubation

Normality test used the *Shapiro-Wilk* result <0.05 p value, it means that the data was not normally distributed to proceed with the non-parametric statistical test that, *Mann Whitney* test. *Mann Whitney* test was used to compare the effectiveness of chitosan gel derived from sewage head of white shrimp (*Litopenaeus vannamei*) against bacterial inhibition *Aggregatibacter Actinobacilus.*

Table 1: Comparison of the Effectiveness of Chitosan Gel as the Inhibition Power of *Aggregatibacter actinomycetemcomitans*

Group	n	Mean ± SD	P.
Chitosan 1% Gel Chitosan 2% Gel	6 6	32.25 ± 1.40 33.90 ± 0.47	0.004
Chitosan 1% Gel Chitosan 3% Gel	6 6	32.25 ± 1.40 35.03 ± 0.48	0.004
Chitosan 2% Gel	6 6	33.90 ± 0.47 35.03 ± 0.48	0.004
Chitosan 3% Gel			

* Significant *Mann-Whitney* test (p <0.05)

Data on Table 1 shows that that, the average power resistor of bacteria Aggregatibacter actinomycetemcomitan that was given gel 1% kitosan was 32.25 mm, with2% gel chitosan was33.90 mm, as well as the provision of chitosan gel 3% showed an increased tendency to 35.03. Statistical analysis showed significant differences inhibition of gel chitosan 1%, gel kitosan 2%, and 3% chitosan gel (p<0.005) against bacteria Aggregatibacter actinomycetemcomitan. The greater the concentration of chitosan gel given, the wider the inhibitory properties of Aggregatibacter actinomycetemcomitan was.

Table 2: Comparison of the Effectiveness of Chitosan Gel with Negative Control on *Aggregatibacter actinomycetemcomitans* Bacteria

Group	N	Mean ± SD	p
Chitosan 1% Gel Negative Control	6 6	32.25 ± 1.40 7.73 ± 0.41	0.003
Chitosan 2% Gel Negative Control	6 6	33.90 ± 0.47 7.73 ± 0.41	0.003
Chitosan 3% Gel Negative Control	6 6	35.03 ± 0.48 7.73 ± 0.41	0.003

* Significant *Man Whitney* Test (p <0.05)

The results in Table 2 shows that the inhibitory zone of chitosan gel was wider compared with negative control, that was only 7.73 mm. Statistical analysis showed that there were significant differences inhibition of gel chitosan 1 %, 2%,3% against the *Aggregatibacter actinomycetemcomitans* bacteria compared with negative controls (p <0.05).

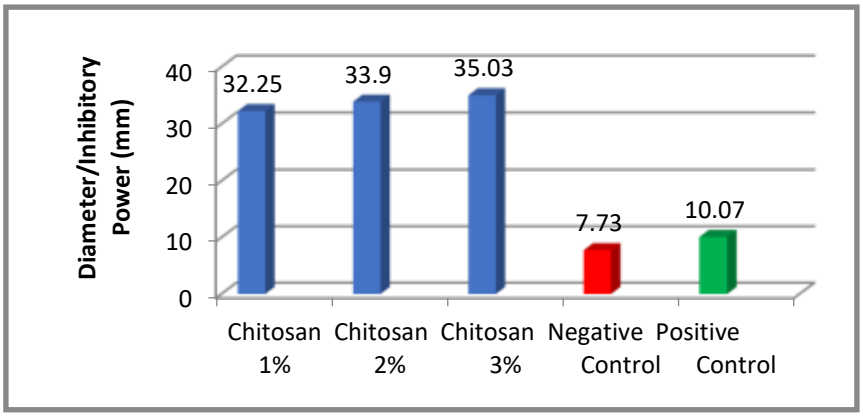
Table 3: Comparison of the Effectiveness of Chitosan Gels with Positive Control of *Aggregatibacter actinomycetemcomitans* Bacteria.

Group	n	Mean ± SD	p
Chitosan 1% Gel Positive Control	6 6	32.25 ± 1.40 10.07 ± 0.76	0.004
Chitosan 2% Gel Positive Control	6 6	33.90 ± 0.47 10.07 ± 0.76	0.004

Chitosan 3% Gel Positive Control	6 6	35.03 ± 0.48 10.07 ± 0.76	0.004

* Significant *Mann Whitney* test (p <0.05)

The data in Table 3 shows that the inhibitory zone of 1%, 2%, and 3% of chitosan gel was much wider compared with a positive control that was only 10.7 mm. Statistical analysis showed that there were significant differences in inhibition of 1%, 2%, and 3% of chitosan gel to Aggregatibacter actinomycetemcomitans bacteria compared with the positive control (p < 0.05).



Graph 1: Comparison of the Effectiveness of Chitosan Gel on *Inhibition of Aggregatibacter actinomycetemcomitans*

Graphic. 1 illustrates the effectiveness of chitosan gel on the calp waste white shrimp (Litopenaeus vannamei) against bacteria Aggregatibacter actinomycetemcomitans. Resistor powerwas characterized by a clear inhibition zone on a paper disk in each replication. The graph above shows that the higher the concentration of the chitosan gel, the greater the area of the clear inhibition zone.

2. Research Results of Clinical and Histological Test of Chitosan Gel on Wound Healing

The research on the potential application of chitosan gel waste of white shrimp head (*Litopenaeusvannamei*) on wound healing in white mice (*Mus musculus*) showed the number of PMN cells and fibroblasts, also measurement of wound healing length in the backs of white rats. The results were analyzed with normality test *of Shapiro-Wilk test* obtained p > 0.05 value, it means that the data was normally distributed then, it could proceed with Statistical parametric *Paired sample t-test* and *Independent Sample t-test*.

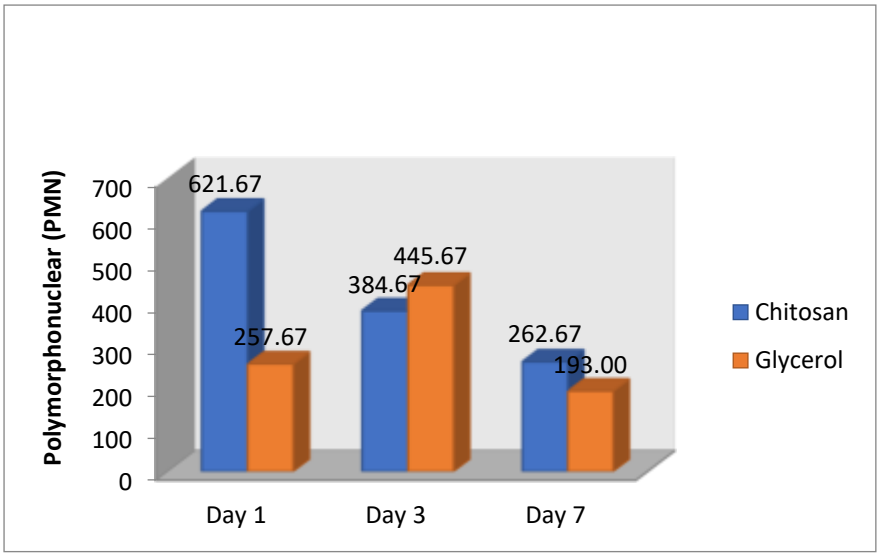
Table 4: Comparison of PMN Cells Given the Application of White Shrimp Head Waste Chitosan Gel (*Litopenaeusvannamei*) and Control (Glycerol) in White Rat Back Injuries (*Mus musculu*)

	Group	N	Polymorphonuclear cells (Mean ± SD)	P.
PMN Day 1	Chitosan Glycerol	3 3	621.67 ± 121. 73 257.67 ± 25. 79	0. 007 *
PMN Day 3	Chitosan Glycerol	3 3	384.67 ± 358. 68 445.67 ± 110. 07	0. 792
PMN Day 7	Chitosan Glycerol	3 3	262.67 ± 33. 65 193.00 ± 10.39	0. 027 *

* Independent sample t-test (p <0.05).

Table 4 shows the comparison where in the first day, the mean cells of polymorphonuclear after administration of chitosan were 621.67, while in the control group (glycerol), Polimorfonuklear cell number was lower, at 257.67. Also, on the 7th day, the average cell of polymorphonuclear after administration of chitosan was 262.67, in the control group (glycerol) the number of polymorphonuclear cells was lower, at 193.0. The results of the *independent* statistical sample *t-test* showed that on day 1 and day 7 there were significant differences in the number of cells Polimorfonuclear in the back injury of white rats (*Litopenaeusvannamei*) in the treatment (chitosan) and

control (glycerol) groups with p-value <0.005. It was seen that the number of Polymorphonuclear cells in the back wounds of white rats (Litopenaeusvannamei) by giving chitosan gel was higher compared with glycerol administration



Graph 2: Comparison of PMN Cells Average Given Application of Chitosan Wastewater Gel Head of White Shrimp (Litopenaeus vannamei) and Control (Glycerol)

Table 5: Comparison of the number of Fibroblast cells given the application of chitosan gel waste of white shrimp head (*Litopenaeusvannamei*) and control (glycerol) in the back injury of white rats (*Mus musculus*)

	Group	n	Fibroblast cells (Mean ± SD)	P.
Day 1	Chitosan Glycerol	3 3	39.00 ± 20. 0 38.67 ± 8.14	0. 948
Day 3	Chitosan Glycerol	3 3	68.33 ± 39.33 39.33 ± 4. 72	0. 087
Day 7	Chitosan Glycerol	3 3	97.33 ± 18. 23 76.33 ± 16.44	0. 312
Day 7	Chitosan Glycerol	3 3	97.33 ± 18. 23 76.33 ± 16.44	0. 312

* Independent sample t-test (p <0.05).

The data in Table 5 shows that the comparison of day 1, the average of Fibroblast cells in chitosan administration was 39.0, in the control group (glycerol) the number of Fibroblast cells was lower, at 38, 67. Thus, on day 3 and day 7 it showed that the average Fibroblast cells after chitosan administration tended to be higher than glycerol administration. Statistical test results of *independent sample t-test* showed no significant differences of cell number of Fibroblast in the back wound of white mice (*Mus musculus*) in the treatment group (chitosan) and control (glycerol) on day 1, day 3 and day 7 (p value> 0.05). It could be seen that the number of Fibroblast cells in the back wounds of white mice (*Mus musculus*) after giving chitosan gel was higher than glycerol administration.

Graph 3. Comparison of Average Fibroblast Cells Given Application of Chitosan Gel Waste from White Shrimp Head (Litopenaeus vannamei) and Control (Glycerol)

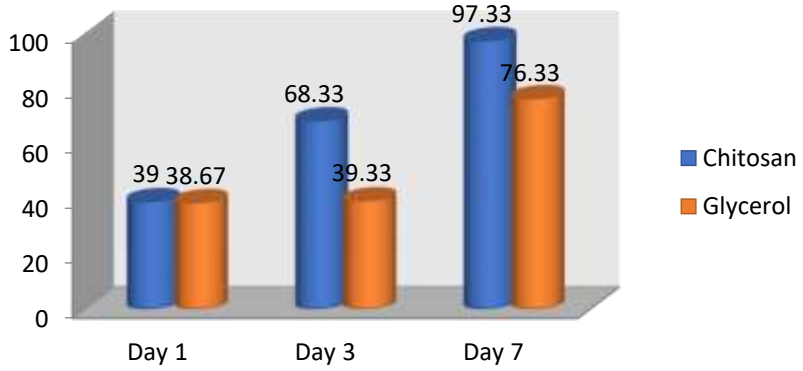


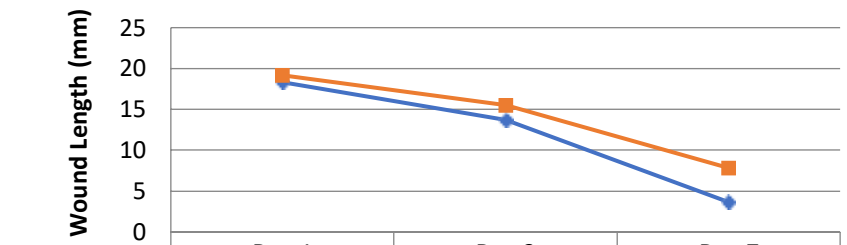
Table 6: Comparison of Wound Length is given the application of chitosan gel waste of white shrimp head (*Litopenaeusvannamei*) and control (glycerol) in the gingival mucosa of the oral cavity of white rats (*Mus musculus*)

	Group	n	Wound Length (mm) (Mean ± SD)	P.
Day 1	Chitosan Glycerol	3 3	18.33 ± 0. 29 19.17 ± 0.29	0. 024 *
Day 3	Chitosan Glycerol	3 3	13.67 ± 1.26 15.50 ± 0. 50	0. 079
Day 7	Chitosan Glycerol	3 3	3.67 ± 0.76 7.83 ± 1.36	0. 008 *

* Independent sample t-test (p <0.05).

The results in Table 6 shows that the comparison on day 1 of the mean wound on chitosan administration was 18. 33 mm, while in the control group (glycerol) wound length was 19, 17 mm. Also, on day 3 and day 7, it was found that the average cuts in the provision of chitosan tend to be shorter than that of glycerol. The results of the *independent sample t-test* showed that there were significant differences in wound length on the backs of white rats (*Mus musculus*) in the treatment (chitosan) and control (glycerol) groups on day 1, and day 7 (p value < 0.005). It could be seen that the average length of the wound on the back of white rats (*Litopenaeusvannamei*) after giving chitosan gel was shorter than glycerol administration.

Graph 4. Comparison of Wound Length (mm) Given by Application of Chitosan Gel Waste of White Shrimp Head (Litopenaeus vannamei) and Control (glycerol)



	Day 1	Day 3	Day 7
Chitosan	18.33	13.67	3.67
Glycerol	19.17	15.5	7.83

DISCUSSION

Chitosan is a natural ingredient compound *bio - polyaminosaccharid* produced from the alkaline deacetylation of chitin, a component of the shell or shell of crustaceans such as crabs, shrimp, lobster, and fungi such as *aspergillus* [13]. Chitosan is a material that has good biodegradability and is non-toxic, biocompatibility, polyelectrolytes, antimicrobial, an anti- inflammatory which supports wound healing, hence these materials are commonly processed or used as new materials that benefit both in medical field as an antimicrobial in clinical applications and in other fields [14].

Kitosan use as an antibacterial is based on the initial interaction between kitosan and electrostatic bacteria. Kitosan has a functional group of amines that are positively charged very strongly, so they can bind to the cell walls of relatively negatively charged bacteria. This bond might occur on the surface of the bacterial cell wall. In addition, -NH2 also has free electron pairs, so this group can attract Ca2 + minerals found in bacterial cell walls by forming coordination covalent bonds [6,7]. The interaction causes permeability changes of the bacterial cell wall, causes an imbalance of pressure in internal cells result intracellular electrolyte leakage, hence the bacterial cell will experiencelysis[15]. The antimicrobial relationship between chitosan and cellular characteristics of bacterial walls found that polysaccharides which have bactericidal and bacteriostatic properties will have better action on gram-negative than gram-positive, due to the composition of phospholipids and carboxylic acids from bacterial cell walls [16]. Chitosan has the potential to be used as an antibacterial agent because it contains *lysosim enzymes* and *aminopolysacharides, which* can inhibit bacterial growth [17].

The effectiveness of chitosan in wound healing, histologically shows a picture of increased inflammatory cell infiltration, vascularity, epithelial cell density, fibroblasts, granulation tissue, wound contractions, and collagen fibers. The mechanism of wound healing consists of four phases: hemostasis, inflammation, proliferation, and remodeling, which takes place through a series of interdependent stages, where cellular components and the matrix act together to rebuild the integrity of damaged tissue and replace lost tissue [18].

The inflammatory process occurs rapidly in the first week, reaching its peak on the 5th day since the injury [19]. If there is no significant contamination or infection, the inflammatory phase is short. Polymorphonuclear (PMN) is the first cell that leads to the site of injury. The number is increasing rapidly and reaching its peak in 24-48 hours. Its main function is to phagocyte the incoming bacteria. In normal wound healing, it seems that the presence of these cells is not so important because wound healing can occur without the presence of these cells. The existence of these cells indicates that the wound is contaminated with bacteria. If there is no infection, the PMN cells are short-lived and the number declined rapidly after three days [18,20]. The administration of chitosan increases the function of PMN and then triggers the next phase of proliferation, [21,22]. Meanwhile, in the control group, there was an increase in the number of PMN cells on the 3rd day more than the chitosan gel treatment group. The condition of in consistency in the number of PMN cells in the control group explained that glycerol did not have anti-bacterial and anti-inflammatory properties so that a significant infection occurred on the 3rd day and the wound healing process was hampered.

Significant decrease in the number of inflammatory cells after day 3 is followed by an increase in the number of fibroblasts, new blood vessels, and collagen, which is called granulation tissue (proliferation phase) [23]. During this phase epithelialization and the formation of new connective tissue occur [19]. During the wound healing stage, angiogenesis plays a role in supplying food and oxygen in the wound area and increases the formation of granulation tissue. The main growth factor in angiogenesis is *vascular endothelial growth factor* (VEGF) produced by dothelial cells and fibroblasts. Fibroblasts themselves play a role in the synthesis, deposition, and remodeling the extracellular matrix. After migrating to the location of wounds, fibroblasts begin downloading the synthesis of the extracellular matrix.

Chitosan has the ability to increase the half-life of the *basic Fibroblast Growth Factor* (bFGF) than the control group by providing protection from being degraded by heat or the influence of enzymes. FGF-2 plays an important role in the development of granulation tissue, fibroblast proliferation, epithelial cell proliferation and angiogenesis [24].

Some cytokines and *growth factors* that play an important role in the process of epithelialization of wounds are from the EGF family, namely EGF (*Epidermal Growth Factor*) and HB-EGF (*Heparin Binding EGF*); FGF family, namely KGF (*Keratinocyte Growth Factor*); and TGFβ1 (*Transforming Growth Factor β1*). EGF has a role to stimulate the proliferation of keratinocytes and to stimulate the release of keratinocyte hemidesmosomes. HB-EGF plays a role in keratinocyte migration in the early phase of reepithelialization. KGF has a role in stimulating the proliferation and migration of keratinocytes. Whereas TGFβ1 plays a role in the proliferation of keratinocytes in the final phase of epithelialization. Chitosan then stimulates the migration of inflammatory cells to the injured area and increases the proliferation of inflammatory cells in the injured area. The increasing proliferation of inflammatory cells causes more cytokines and *growth factors* to be released by these inflammatory cells. Cytokines and *growth factors* that cause the migration and proliferation of keratinocytes which is dominant in the epithelialization of cells so the process can happen faster epithelialization and the wound can be immediately shut perfectly[25,26].

The ability of chitosan head waste white shrimp (Litopenaeusvannamei) to accelerate wound healing in the treatment group is due to the ability of chitosan to inhibit the release of arachidonic acid and the release of lysosomal enzymes from the membrane by blocking the cyclooxygenase pathway. The ability of chitosan to inhibit the cyclooxygenase and lipoxygenase enzymes in inflammation causes the production of prostaglandins and leukotrin to be reduced. Emphasis prostaglandins and leukotrienes as mediators of inflammation may lead to a reduction in pain in the swelling, reduce the occurrence of vasodilatation and local blood flow so that the decrease

might constellation of inflammatory cells in the wound area. Thus, the inflammatory phase lasts briefly and enters the phase of uninterrupted proliferation [27 - 31].

CONCLUSION

Chitosan gel of white shrimp head waste *(Litopenaeusvannamei)* have inhibitory effect to *Aggregatibacter actinomycetemcomitans* bacteria and accelerate the wound healing of white mouse back (*Mus musculus*), accompanied by a decrease in total of polymorphonuclear cells and increase in fibroblasts cell.

SUGGESTION

With the development of science in terms of biomaterials in the medical field, it is expected to be able to deeply develop the benefits of chitosan, not only as an antimicrobial potential but also to the dentistry-related field, finally new and safe biomass can be developed.

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