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BIOFILM CHITOSAN AS MODERN DRESSING FOR ULCERS

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ABSTRACT

Modern dressing techniques as open wound dressings are still effective and suitable for use, especially for people with open wounds such as ulcers, but they still has disadvantages, such as the expensive prices and needs for other antibiotics to prevent inflammation. Previous studies reported an increase in the number of antibiotic resistance, which sparked the idea of producing new dressing materials that have strong antimicrobial and biocompatible properties. The material suitable for the idea of a wound dressing is chitosan biofilm because it has strong antibacterial properties and has a similar structure to the skin tissue. This study aims to produce chitosan biofilm using the deacetylation method using a strong base. The physicochemical characterization results of biofilms showed a deacetylation degree of 87.13 with a voltage of 1.15 ± 0.00 and a polycationic group of biofilms that appeared at a wave number of 2.1714 ± 0.0000 nm. From the measurement of the antibacterial power of chitosan biofilm against skin surface bacteria, the inhibition zone diameter was 18.93 ± 0.12; 19.50 ± 0.17; 20.20 ± 0.23; 20.13 ± 0.03 and 22.53 ± 0.09 against S. aureus, S. epidermidis, P. aeruginosa, E. coli, Bacillus sp. Overall, it can be concluded that biofilm chitosan has the opportunity to be applied as a dressing in wound care.

Keywords: *Antibacterial, Biofilm, Chitosan, Ulcers*

INTRODUCTION

A wound is a broken off of tissue continuity or damage to the structure and anatomy of the skin due to injury or surgery [1]. One example of a wound that requires wound care with that proper technique is an ulcer. Diabetic foot ulcer often occur in the lower limb are at risk of causing amputation [2]. Ulcer are open wounds on the skin surface, mucous membrane, mortality of extensive tissues and accompanied by invasive saprophytic bacteria. Diabetic *Ulcers* is usually experienced by patients with prolonged bed rest such as stroke patients. *Ulcers* occur on areas of the skin that have been subject to prolonged pressure such as the back, calf or heel. Decubitus *ulcers* are at risk of causing infection to sepsis [4].

The principle of ulcers treatment is to maintain moisture and barrier of the tissues. The Moisture of skin could support the wound recovery [5]. One example of bandage used at the present is modern dressing. According to the previous study, the modern

dressing techniques are effectively for dressings especially for diabetics ulcer than conventional techniques which taken a long time recovery [3],[6]. However, modern dressing also have weaknesses such as the price of treatment is quite expensive, the tools and materials are exclusively on pharmacy or under doctors order can applied by paramedic only [7].

The weakness of modern dressing are initiate many nurses to applied antibiotics as an alternative treatment of diabetic ulcer such as erythromycin. Unfortunately, The regular use of topical antibiotics such as erythromycin, tetracycline and ciprofloxacin are causes of resistance. Based on data, the bacteria on 12.05% patient with diabetic foot ulcer, are not inhibited by antibiotics [8]. This cases showing that the application of antibiotics for long treatment of diabetic foot ulcer are not effectively

The increasing cases of antibiotics resistant are initiate to improvise the utilize of natural compound for ulcers treatment. This materials also having an antimicrobial effect to inhibit bacterial causes of inflammation in ulcer. The natural compounds is chitosan. This bioactive compound can prevent the spread and cloning of bacterial by damaging the cell membrane of the bacteria [9]. Chitosan is an exoscheletal of crustacean shells that causes of active metabolite on tissues which is an antibacterial for pathogen microorganism such as *E. coli*, Vibrio cholerae, Shigella dysenteriae and Bacteroides fragilis [10]. According to previous researcher, chitosan is a natural biopolymer derived from chitin, the main component of external crustaceans such as shrimp and crab shells [11]. Some studies shown that chitosan is effective in rapidly recovering wound skin because it has specific physicochemical characteristic such as bioactive, biocompatible, anti-bacterial, anti-fungal and biodegradable. Chitosan has been used for bacteriostatic, immunology, anti-tumor, cicatrizant, homeostatic and anticoagulant, ointments for wounds, eye medicine, orthopedics, and surgical suture recovery [12]. The aim of this study are to made chitosan biofilm as a modern dressing that could be apply for diabetic foot ulcers in the next research and prove the antibacterial effect of Chitosan.

METHOD

Nano chitosan biofilm research was conducted at the Universitas Kristen Satya Wacana (UKSW) FKIK Lab. The chitosan is derived from the shells of tiger shrimp (*Panaeus Monodon*). This is a waste product that can be easily found in traditional market in Salatiga, Central Java. Some tools were used in this study such as knife, spatula, magnetic stirrer, acid bath, thermometer, viscometer and UV-VIS spectrophotometer. The chemicals used are hydrochloric acid (HCl), acetic acid (vinegar), NaOH, and NaPO4, all of which are in the Pro-Analysis (PA) category.

1. Synthesis of Chitin from Shrimp Shells

Shrimp shells obtained from the market are washed with H_2O_2 and dried for 3 hours using the oven at 80^{o}C. After drying, shrimp shells are moderated in 500 mL of HCl 5% (v/v) for 24 hours to remove minerals. After 24 hours, the shrimp shells are washed

to a neutral pH and dried. After drying, maceration of shrimp shells continued using 500 mL of NaOH 4% (w/v) for 24 hours to remove protein and fat from shrimp shells. After 24 hours, a pure white chitin polymer sheet was obtained, chitin was then rinsed to neutral pH and then dried [13].

2. Synthesis of Chitosan from Chitin

Chitin obtained from the shrimp shells was macerated in 70% (w / v) NaOH solution with a ratio of 1g of sample in 25 mL of 70% NaOH. Maceration is carried out at a temperature of 120-130oC for 90 minutes to eliminate acetamin groups from chitin. After 90 minutes, a yellowish white polymer chitosan was obtained. Chitosan is washed and rinsed to neutral pH then dried, to qualitatively test the formation of chitosan, 0.5g of chitosan is taken and then dissolved in 5 mL of 1-5% (v / v) acetic acid if a gel is formed, it means that the polymer obtained is chitosan and deacetylation is successful [13]

3. **Synthesis of Nano Chitosan**

Take 10 ml of 1% chitosan solution and then drop it with 10% phosphoric acid to form a fine white precipitate, the precipitate is then separated from the solution and dried (Kurniasih and Dwi, 2011; Mardliyanti *et al.* 2012; Komariah, 2015).

4. Chitosan Biofilm Making

Taken 10 ml of Chitosan gel and poured into a mold container measuring 5 x 2 cm^2 and mold container then flattened and dried at room temperature. After drying, the biofilm can be removed from the mold. For large scale needs, chitosan gel can be poured on a large glass plate and then dried and cut to the size needed.

5. Use in Wound Handling

Mold container then flattened and dried at room temperature. After drying, the biofilm can be removed from the mold. For large scale needs, chitosan gel can be poured on a large glass plate and then dried and cut to the size needed.

RESULTS AND DISCUSSIONS

Shrimp shells is use as chitosan synthesis material because the abundance of shrimp shells in Indonesia is very large and can be obtained at a relatively cheap price. In shrimp shells, natural biocontrol of the active ingredient chitin/chitosan was found [14]. Shrimp production capacity in Indonesia reaches 500.000 tons per year by producing around 300.000 tons of waste from 170 units of shrimp processing industry [15]. The results of the shrimp shell characterization are show in Table 1.

Table 1. GraphmetricAlly Measured Mineral Content in Shrimp Shells

Parameter	Ingredients InX (± SE):			
	Shrimp Shells	Chitin	Chitosan	Chitosan TPP
Water (%)	0.1167 ± 0.0082	0.10 ± 0.00	0.10 ± 0.00	-
Ash (g/g)	0.0071 ± 0.0003	0.0059 ± 0.0002	0.0057 ± 0.0001	-
SiO (g/g)	0.0023 ± 0.0001	0.0014 ± 0.0002	0.0013 ± 0.0001	-
Organic Matter (g/g)	0.9929 ± 0.0003	0.9941 ± 0.0002	0.9943 ± 0.0001	-
Organic Carbon (g/g)	0.5759 ± 0.0002	0.5766 ± 0.0001	0.5767 ± 0.0001	-

Parameter	Ingredients InX (± SE):			
	Shrimp Shells	Chitin	Chitosan	Chitosan TPP
N Total (%)	11.05 ± 0.0120	2.72 ± 0.0797	0.25 ± 0.0203	-
Fat (%)	5.31 ± 0.1747	2.20 ± 0.1000	0.06 ± 0.0067	-
PO_4 (g/g)	0.0942 ± 0.0023	0.0870 ± 0.0006	0.0205 ± 0.0002	0.0896 ± 0.0001
P_2O_5 Available (g/g)	0.0052 ± 0.0002	0.0049 ± 0.0001	0.0035 ± 0.0002	0.0055 ± 0.0003
Dissolved P_2O_5 (g/g)	0.0025 ± 0.0001	0.0022 ± 0.0001	0.0017 ± 0.0001	0.0022 ± 0.0001
P_2O_5 Total (g/g)	0.0071 ± 0.0002	0.0075 ± 0.0001	0.0043 ± 0.0001	0.0076 ± 0.0001
Total P (g/g)	0.0593 ± 0.0020	0.0219 ± 0.0002	0.0054 ± 0.0002	0.0421 ± 0.0005
Deasetilasi (%)	-	18.05	87.13	66.22
Rendemen (%)	-	10.52 ± 0.0233	79.59 ± 0.0504	54.12 ± 0.0203

The N content of 11.05 ± 0.0120 in shrimp shells indicates that shrimp shells can be used as a source of synthetic chitosan which has a great opportunity in its application. The synthetic yield of shrimp shells also showed a fairly large chance of success, namely 79.59 ± 0.0504. Furthermore, physicochemical characterization of synthetic chitosan from shrimp shells was carried out to determine the chemical properties of chitosan as the first step in applying chitosan as an antibacterial active ingredient. The results of chitosan characterization are shown in Table 2.

Table 2. Characteristics of Chitosan and Chitosan Treatment Results

Products	Solubility(Brix %)	Density (g/cc)	Viscosity			MW (g/mol)	Z-Potential (Electricity		
			Absolute (P.a)	Kinetics (Cts)	Dynamic (CPs)		Voltage (mV)	Current Strength (mA)	Electrical Resistance (Ohm)
Chitosan Gel	5.1 ± 0.00	1.1251 ± 0.0005	2.10 ± 0.0000	1.87 ± 0.0009	4.76 ± 0.0000	2.1714 ± 0.0000	1.15 ± 0.00	20 ± 0.00	62 ± 0.00
Chitosan 1%	10.13 ± 0.03	0.8808 ± 0.0112	1.10 ± 0.0577	1.25 ± 0.0790	9.14 ± 0.4818	1.1374 ± 0.0597	1.20 ± 0.00	50 ± 0.00	54 ± 0.00
Chitosan TPP	1.2 ± 0.00	0.9967 ± 0.0036	1.50 ± 0.0000	1.51 ± 0.0055	6.67 ± 0.0000	1.5510 ± 0.0000	2.70 ± 0.00	35 ± 0.00	54 ± 0.00

Based on the results the chitosan Tripoliphosphate (TPP) has an adhesive, flexible and antibacterial power that is stronger characteristics than chitosan gel and 1% chitosan. The Application of chitosan on the wounds skin greatly affects the healing process, with the prevent the initial symptoms of inflammation marked by damage of tissue and blood vessel, increasing excretion of platelets and blood clots (blood clots) which are increasing recovering process wound skin barrier [16]. The previous study, also state that application of chitosan can trigger macrophage cells to increase the production of growth factors such as PDGF, FGF and TGFβ so that they can accelerate the process of fibroblasts in proliferation, migration and forming an extracellular matrix [17]. The increasing number of fibroblast cells, the more collagen produced will also increase. Therefore, the process of synthesis and collagen degradation can be balanced. In

2009, some researcher, present that chitosan has great potential to be used as an antibacterial agent, because it contains lysozyme enzymes and aminopolysacharida groups which can inhibit bacterial growth by inhibiting chitosan in bacteria [18]. The nine years later, another researcher find that shrimp shells chitosan is effective as an antibacterial which can inhibit the growth of the bacteria *Staphylococcus epidermidis Pseudomonas eruginosa, Propionibacterium agnes,* including *E. coli* [18].

The purpose of diabetic ulcer treatment is to prevent the patient from inflammation and save the patient from amputation. The Chitosan Initiated the recovery of ulcer rapidly because the ability to absorb the water 500mg/ml (Islam et al) can keep the surface still in dry-moisture condition not totally moisture, the low viscosity (Islam et al) can penetrate the skin cells made an ionic crosslink to reconnect (repair) the skin and protein tisues.

The inflammation (in the wound and ulcer) majority caused by the activity of S. aureus and P. aeruginosa bacteries. This activity symphtomized by the appearance of the white-yellow spot around the ulcer. By inhibiting the activity of S. aureus, the application of chitosan can be new approach to prevent inflammation for diabetic foot ulcer.

Chitosan is Osteoconductive, bioactive which can improve the wound recovery process. Then, Chitosan has been widely used in the medical field because it has antimicrobial properties that make it attractive to use as a bioactive coating. Chitosan also triggering the tissue growth when combined with calcium phosphate compounds [19].

Table 3. Antibacterial Power of Chitosan and Chitosan TPP (Nano) Based on Average Diameter of Inhibitory Zone (DIZ)

	Diameter of Bland Area (DIZ) (mm) Against Bacteria				
	S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. colli*	*Bacillus sp*
Chitosan Gel	18.93 ± 0.12	19.50 ± 0.17	20.20 ± 0.23	20.13 ± 0.03	22.53 ± 0.09
Kitosan TPP	17.23 ± 0.19	20.47 ± 0.08	16.40 ± 0.26	20.43 ± 0.12	21.53 ± 0.19
Tetracycline	21.23 ± 0.15	21.00 ± 0.00	23.93 ± 0.15	22.67 ± 0.19	25.67 ± 0.19
Streptomycin	21.47 ± 0.12	21.13 ± 0.03	20.87 ± 0.03	21.07 ± 0.19	21.87 ± 0.03
Erythromycin	22.77 ± 0.07	22.80 ± 0.06	23.30 ± 0.10	25.90 ± 0.17	26.60 ± 0.10

The table above proves that chitosan has strong antibacterial resistance against bacteria on the ulcer. Antibacterials are needed on wounds to get rid of bacteria because they can cause infection. The bacteria that most often produce pus (pus) are *Staphylococcus aureus,* Klebsiella spp [20]. The ointment with the active ingredient Curcuma aeruginosa has a diameter (14.52 mm), C.longa L (13.26 mm) and C.xhantoriza L (7.89 mm) [21]. This proves that the inhibitory power of chitosan is much greater than the active ingredients of kencur, turmeric and temugiring.

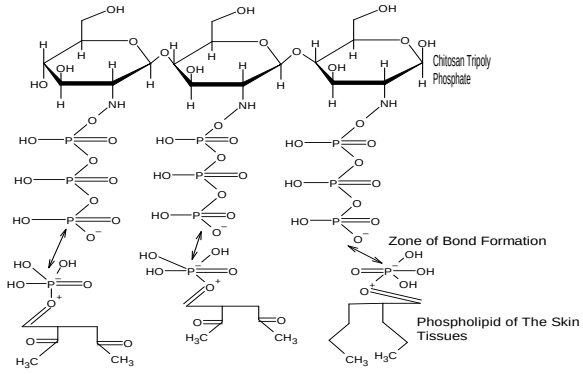

Figure 1. In vitro Biofilm
Wadaniati and Sugiyanti (2009)

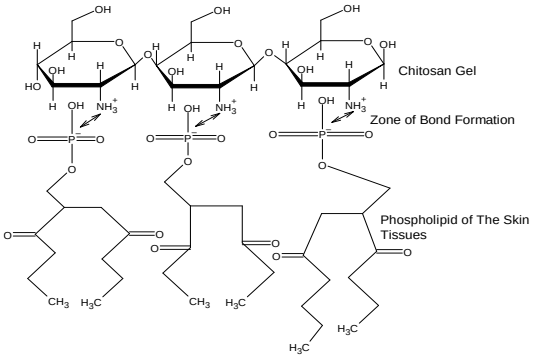

Figure 2. In vitro Biofilm
Islam *et al* (2011)

The characterization results in the in vitro biofilm image above show that there is a tug-of-war interaction between the polycationic group of chitosan and phospholipids in the skin tissue to produce protein fiber joints that cover the wound.

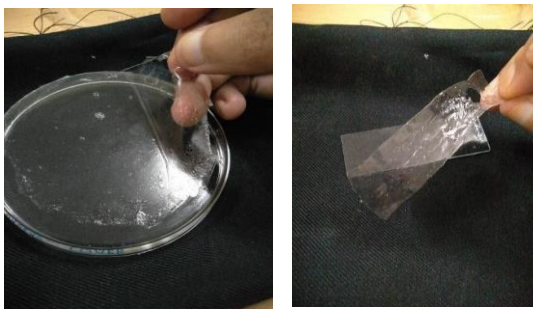

Figure 3. Biofilm Chitosan Images
(Author Documentation)

The chitosan biofilm image above shows that chitosan gel can be used as a biofilm which has a great opportunity as a wound cover in ulcers with antibacterial resistance [22]. Biofilms are designed to be applied to wounds using a bandage. In addition, the resulting biofilm has a clear and transparent color so it is different from other biofilms. Wounds that have been covered by biofilm no need to be cleaned regularly, because the clear and transparent of biofilm makes it easier to see the wound healing process or an infection on the ulcer surface. Therefore, biofilm could be a new in the medical treatment for ulcer.

CONCLUSION

The Chitin synthesis from the shells by demineralitation and deproteination obtained the rendement of Chitin 10.52 ± 0.0233% with deacetilation degree 18.05%.The Chitosan synthesis from Chitin by deacetilation using NaOH 70% (w/v) obtained the rendement of Chitosan 79.59 ± 0.0504% with deacetilation degree 87.13% and Molecular Weight 2.1714 ± 0.0000g/mol. Chitosan-TPP synthesis from Chitosan by ionic gelation using NaTPP 50% (w/v) showed the rendement 54.12 ± 0.0203% and deacetilation degree of Chitosan-TPP 66.22% and Molecular Weight 1.5510 ± 0.0000g/mol. Physicochemical Characterization by measuring the Solubility, Density, Viscosity, Mollecular Weight and Z-Potential showed that the Chitosan and Chitosan-TPP feasible to apply as Biofilm / Biopolymer in Medical applications. Antibacterial activity test against *S. epidermidis, S. aureus, P.aeruginosa, E. colli and Bacillus sp* showed the Chitosan and

Chitosan-TPP have antibacterial activity clasificated in strong activity range.

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