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Cytotoxicity Assay Using Brine Shrimp Lethality Test on Collagen-Chitosan Wond Dressing Sterilized by Ultraviolet Light

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Abstract

Collagen gives a moist state on the wound area to accelerate the wound healing process. Chitosan is a polymer known as non-toxic, antibacterial, antifungal, biodegradable, and biocompatible material. Combination of collagen and chitosan is expected to be the best biomaterials as a wound dressing for the healing process. The study aimed to determine the cytotoxicity assay on collagen-chitosan wound dressing sterilized by ultraviolet (UV) Light using Brine Shrimp Lethality Test (BSLT) method. The test groups were divided into K0, K1, K2, and K3 groups. K0 contained pure chitosan as a control group, K1 contained collagen 25%-chitosan 75%, K2 contained collagen 50%-chitosan 50%, K3 contained collagen 75%-Chitosan 25%. Collagen was extracted from the skin and scalp of snakehead fish (Channa striata), then it was mixed with chitosan until collagen-chitosan wound dressing formed. This study used Brine Shrimp Level Test (BSLT) method with solution concentration: 10, 50, 100, 250, 500, 750 and 1000 ppm. Based on the results, it showed that K0, K1, K2, and K3 group had LC_{50} > 1000, proved that collagen-chitosan wound dressing was non-toxic material. The conclusion of the study explain that composite wound dressing based on collagen-chitosan in all groups that was sterilized under UV-Light for 15 minutes was not toxic. Also, it showed LC_{50} > 1000 based on Brine Shrimp Lethality Test.

Keywords

Collagen, chitosan, BSLT, wound dressing, ultraviolet

INTRODUCTION

There are two kinds of wound dressing, such as traditional wound dressing including gauze, plasters, lint, bandages, cotton wool, and modern wound dressing including films, hydrogel, hydrocolloids, foam and composites (1,2). Wound dressing aplication has main roles in healing the wound and preventing infection on wound area. Best wound dressing with good quality could accelerate the wound healing, prevent the wound from infection of bacteria and fungal, and reduce pains (2). The particular wound dressing can promote a newly epithelium

layer that may not be damaged during the dressing removal around the wound (3). The composite dressing is made from a combination of any kind of materials for dressing. It is suitable to burn wounds, surgical wounds, infectious wound and refractory chronic wound (2).

Collagen and chitosan are known as biomaterials that have bioproperties including biocompatible and biodegradable (4). Therefore, it can be developed into composite dressing for wound healing. However, its application is limited due to clinical test before using for human. Few steps were needed before clinical test of a wound dressing which are toxicity (5), quality (6), in vitro and in vivo evaluation test (7).

Based on Ariyadi and Dewi (8) study, it showed that dry sterilization using ultraviolet (UV) irradiation for 10 and 15 minutes would not be contaminated by Bacillus subtilis colony (8). Therefore, this research used composite collagen-chitosan extracted from *Channa striata* collagen that was sterilized under ultraviolet (UV) light for 15 minutes to determine LC_{50} using Brine Shrimp Lethally Test (BSLT). Previously, the brine shrimp was utilized in various bioassay such as analysis of pesticide residues, stream pollutant, anesthetics, dinoflagellate toxins, mycotoxins, cocarcinogenecity of phorbol esters, and toxicants in marine (9).

MATERIALS AND METHODS

Skin and scales of sneakhead fish were collected from Srikandi fishmonger at Banjar Asri Village, Tanggulangin Sub-District, Sidoarjo, East Java Province, Indonesia. The 100 mesh Chitosan powder (food and medical grade) was made from black tiger shrimp shells and it was obtained from Monodon (Marine Natural Product). The cytotoxicity assay was conducted by using *Artemia salina* (Golden West Artemia, Supreme Plus).

The collagen was extracted from the skin and scales of sneakhead fish by macerating it in HCl 2% for 48 hours and it was neutralized with NaOH 1M. Chitosan powder was dissolved in CH_3COOH 1% (4). Collagen-chitosan composite was synthesized by mixing collagen 25% : chitosan 75% as K1 group; collagen 50% : chitosan 50% as K2 group; collagen 75% and chitosan 25% as K3 group; and pure chitosan as a control group (K0).

The cytotoxicity assay was carried out with the Brine Shrimp Lethally Test (BSLT) by using *A. salina*. Each collagen-chitosan composite of K0, K1, K2 and K3 was dissolved with DMSO 1 % then was divided into 10 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm. As much as 5 mL of each concentration was added into petri dish then it was added with 5 ml of seawater contained in ten *A. salina*. Hereafter, it was incubated for 24 hours (10).

$$\% \text{ Mortality } = \frac{\text{Total Larvae Mortality}}{\text{Total Larvae}} \text{ x } 100\ \%$$

LC_{50} assessment was analyzed by Microsoft excel with Probit analysis. Afterward, the toxicity categories of each sample could be determined based on the Table 1.

Table 1. LC_{50} categories (9)

Categories	LC_{50} (ppm)
Very Toxic	<30
Toxic	30-1000
Non-Toxic	> 1000

(Source: Meyer *et al*, 1982)

RESULTS

Brine Shrimp Lethally Test (BSLT) was conducted using *Artemia saline* larvae to study the toxicity of collagen-chitosan composite. This study was a preliminary test before the composite applied *in vivo*. The result of probit analysis of larvae mortality percentage for each group sample can be seen in Table 2, 3, 4, and 5.

Table 2. Percentage of Larvae Mortality in K0 Group

Concentration (ppm)	Log 10	Mortality	Total Larvae	%Mortality	Probit
10	1,00	0	10	0%	-
50	1,70	3	10	30%	4,48
100	2,00	0	10	0%	-
250	2,40	0	10	0%	-
500	2,70	1	10	10%	3,72
750	2.88	0	10	0%	-
1000	3,00	0	10	0%	-

Table 3. Percentage of Larvae Mortality in K1 Group

Concentration (ppm)	Log 10	Mortality	Total Larvae	%Mortality	Probit
10	1,00	0	10	0%	-
50	1,70	0	10	0%	-
100	2,00	1	10	10%	3,72
250	2,40	2	10	20%	4,16
500	2,70	1	10	10%	3,72
750	2.88	0	10	0%	-
1000	3,00	2	10	20%	4,16

Table 4. Percentage of Larvae Mortality in K2 Group

Concentration (ppm)	Log 10	Mortality	Total Larvae	%Mortality	Probit
10	1,00	3	10	30%	4,48
50	1,70	1	10	10%	3,72
100	2,00	0	10	0%	-
250	2,40	2	10	20%	4,16
500	2,70	2	10	20%	4,16
750	2.88	2	10	20%	4,16
1000	3,00	0	10	0%	-

Table 5. Percentage of Larvae Mortality in K3 Group

Concentration (ppm)	Log 10	Mortality	Total Larvae	%Mortality	Probit
10	1,00	2	10	20%	4,16
50	1,70	0	10	0%	-
100	2,00	0	10	0%	-
250	2,40	0	10	0%	-
500	2,70	1	10	10%	3,72
750	2.88	3	10	30%	4,48
1000	3,00	1	10	10%	3,72

Tabel 6. LC_{50} Assesment

No.	Group	LC_{50}	Toxicity
1	K0	> 1000	Non-Toxic
2	K1	> 1000	Non-Toxic
3	K2	> 1000	Non-Toxic
4	K3	> 1000	Non-Toxic

(Source: Meyer *et al*, 1982)

Based on the results, it showed that collagen-chitosan composite sterilized by UV light was not toxic. This is based on the interpretation of the data, according to Meyer et al, (9).

DISCUSSION

The composit dressing is used for wound treatment to reduce the contamination risk. The research about wound dressing had developed rapidly due to the demand for wound treatment which increase every year. Chitosan and collagen are popular biomaterials for wound dressing because of its bioproperties. Sterilization of materials before in vivo assay must be done to prevent microbial and fungal contamination. The UV Light exposure on materials could mantain sterility of materials. However, not every material suitable for this methods. Therefore, cytotoxicity assay has to be conducted to analyse its bioproperties before it used for humans. Brine Shrimp Lethally Test was a

simple cytotoxicity assay by using *A. salina* to obtain LC_{50}. If LC_{50} value is more than 1000, it means that the materials tested were not toxic (9).

Based on the results of the study, it showed that wound dressing for skin wound healing that sterilized by UV light at the whole group was not toxic because $LC_{50} >$ 1000. Chitosan was also known as anti-bacterial and anti-fungal properties (4). Exposure of UV sterilization used was 254 nm to inactivate the microbe. Absorption of UV light on microbe could damage nucleic acids and their constituents that caused mutagenetic effect and cell retardation (11). Ultra-Violet (UV) radiation on the material causes chemical modification of nucleoprotein compounds and occurs the cross-linked of timin molecules that could generate false genetic code, promoting mutation that will cause the damage and weaken the vital functions of the organism until dead (8).

Based on the results of Ariyadi and Dewi (8) study, it showed that the best UV method as anti-bacterial treatment was obtained in 10 and 15 minutes. Therefore, this study was using 15 minute for UV exposure as a sterilization method on the wound dressing. Collagen could heal chronics wound by stimulating fibroblast and promoting endogenous collagen synthesis on wound area (12). Based on Lei et al. (12) research, it showed that collagen hydrogel dressing could promote the rate and quality of diabetic full-thickness wound healing on rats. Moreover, collagen from snakehead fish contains glycine, glutamine and arginine. Glycine has a role in collagen synthesis around the wound area, while glutamine has a role as an energy source during the inflammatory phase and proliferation phase of wound healing. Arginine has a role in immunity system and stimulates endothelial cell function. A chitosan was known as a biomaterial for drug release on wound area due to its hydrogels properties (13). Therefore, the combination of collagen and chitosan can enhance the bioproperties of collagen-chitosan composite dressing.

CONCLUSIONS

Collagen-chitosan based composite wound dressing in all groups (K0, K1, K2 and K3) that was sterilized under UV-Light for 15 minutes were not toxic based on Brine Shrimp Lethally Test which showed $LC_{50} >$ 1000.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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