

SYNTHESIS AND CHARACTERIZATIONS OF ABSORBENT DRESSING TURMERIC EXTRACT CURCUMIN CHITOSAN-ALGINATE HYDROGEN AND ZNO NANO FOR MEDIATE AND HIGH EXUDATION

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Introduction

The dressing is a material used to cover an injury. This cover has a purpose to protect the injury from external infection until the natural curing happens through rubbing with the cloth [1]. The most important thing to look after an injury is by selecting proper injury dressing. The basic principles to take optimal dressing are: a moist dressing for dry injury, a dressing which can absorb fluid easily for injury with large exudate, necrotic nets must be banished if the injury has them and antibiotic for infected injury [2]. Also, the principle of healing the cure is to moisturize it, not to make it wet [3]. Thus, anti-bacteria absorbent dressing is a dressing to absorb exudate injury used to moisturize injury. Conventional absorbent dressing still uses gauze while modern absorbent dressing uses hydrocolloid and sodium-alginate material [4].

Gauze has some influential lacks [5]. One of them is its environmental condition from wet to dry. It makes the cells formed adhere on the gauze so that it causes pain when the gauze is dismissed. Thus, the use of modern absorbent dressing such sodium-alginate could decrease the pain. It happens because the sodium-alginate turns into hydrogel when absorbing the exudate so that it will not adhere to the skin. Besides, the change of sodium-alginate dressing can be done once three to four days because it contains antimicrobial. Different from modern absorbent dressing, the use of gauze must be changed every day to avoid the infection [6]. Unfortunately, the sodium-alginate dressing is got from import, nowadays. Thus, its price is expensive enough. The basic material of sodium-alginate dressing is brown seaweed (*Sargassum sp.*) which is abundantly growing in Indonesia [7].

Chitosan was a chemical substance which was derivate from chitin. Generally, it was isolated from invertebrate's skeleton such *crustacean sp* eggshell of shrimp, lobster, crabs, and others. Chitosan was non-toxic, biocompatible, biodegradable, and polycationic. Further, it can create gel if it contacts with water in a sour situation because of the cross knot happen in the structure [8]. The body will get inflammation process after getting an injury. When the inflammation process is running out, there is a series of reaction which kills dangerous agents and prevents them to spread out. These dangerous agents could be germs, bacteria, microbes and other agents that can hamper the healing process of the injury. These agents can

hamper the healing process of the wound. The optimization process of healing can be helped by adding therapy agents. These agents include substances which have a function to hamper the growth of germs, microbes, fungus, bacteria, and any others. Curcumin is one of therapeutic agent useful for healing an injury.

Curcumin is a substance consisted of turmeric or Curcuma which is anti-inflammation, anti-immunodeficiency, anti-virus, anti-fungus, anti-oxidant, anti-cariogenic and anti-infection [9]. The structure of chitosan and alginate can create ionic content because alginate was pollination and chitosan is pollination. Thus, they will make complex polyelectrolyte if those two materials are mixed. This complex polyelectrolyte can fasten liquid absorbing [10]. The previous study was done by Dai, et.al, showed that the sponge will have bad absorbent power if the alginate is bigger than chitosan as compare to the sponge with more chitosan. This study does not only use alginate curcumin and chitosan as the ingredients but also uses ZnO Nano because it has better antimicrobial activity than large particles. However, the small size particles (less than 1000 nm) and whose surface area allow interaction in treating wounds, reduce inflammation, and anti-microbes [11]. Thus, this research carried out syntactic absorbent dressing alginate chitosan and ZnO nano because it has good biocompatibility properties to absorb fluids, antibacterial activity, and hydrating agent to accelerate wound healing.

Materials and methods

Materials used in this study were sodium alginate got from Franken Chemical (Germany), curcumin PureBulk and chitosan from Merck (Germany), fibroblasts cell for MTT test, Phosphate Buffered Saline (PBS) for ability of absorbing test, equates to dissolve sodium alginate, acetic acid to dissolve chitosan, and ethanol to dissolve curcumin. Further, this study also used some supporting tools, such as Lyophilizer, freezer drying type Lyovapor L-300 from Buchi (Switzerland), trays, magnetic stirrer, centrifuge, digital balance, beaker glass, spatula, and aluminum foil.

Sponge Making Process

Sponge A is made of a mixture of powdered sodium alginate, chitosan, and Nano curcumin and ZnO that have been diluted with a solvent sodium alginate dissolved in equates, chitosan is dissolved in acetic acid, and curcumin is diluted with ethanol. Each emulsion is mixed and stirred with a magnetic stirrer, in order a homogeneous can further be centrifuged to separate the residue and the supernatant. The residue is taken to be a sponge. The residue is then poured into a 0.5 cm rectangular tray. The tray is stored in the freezer with the temperature range of -700C to -900C for \pm 24 hours, and after 24 hours in the freezer, the samples are removed and directly lyophilized for 24 hours with temperature approximately on -1000C and pressure in millitorr [12]. The Composition of Alginate-Chitosan-Curcumin-Nano ZnO in Table 1.

Table 1. Variation of Sponge

Sponge	Curcumin : Chitosan	Alginate: Nano ZnO
A	1:2	Stable
B	0:4	Stable
C	1:4	Stable

Characterization

Fourier transform infrared spectroscopy (FTIR) test

Sodium alginate, chitosan, curcumin, and Nano ZnO that become the primary constituents of making

absorbent dressing sponge is previously tested by the FT-IR absorption area of 4000-400 nm. The results are shown in figure 1, figure 2, and figure 3.

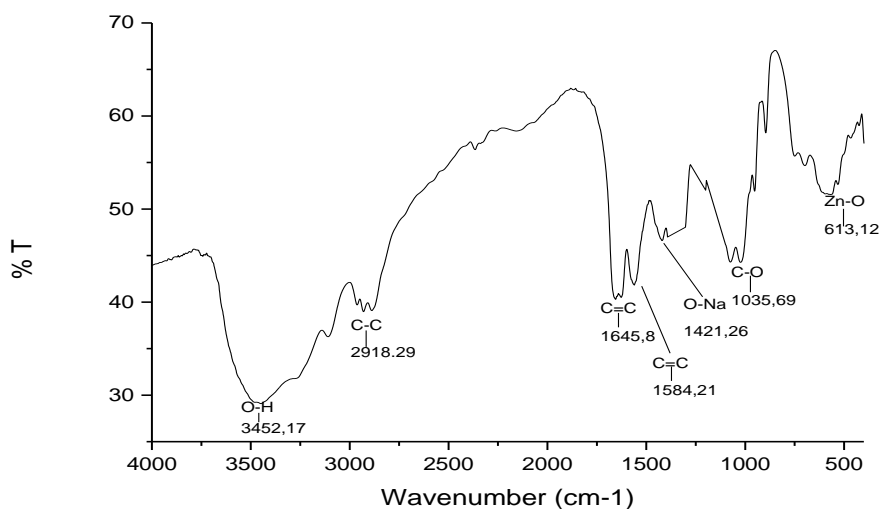


Figure 1. The spectrum FTIR sponge A (Comparison of curcumin: chitosan 1:2)

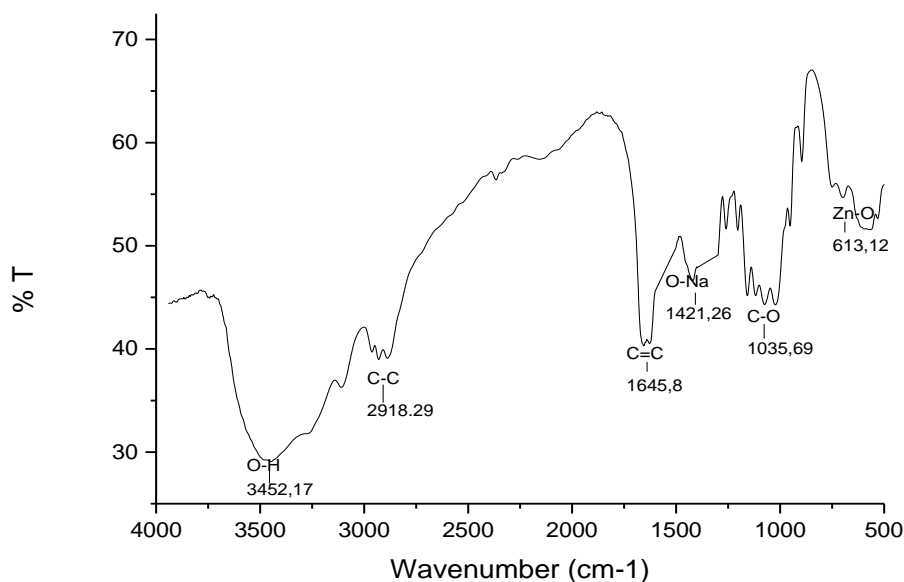


Figure 2. The spectrum FTIR sponge B (Comparison of curcumin:chitosan 0:4)

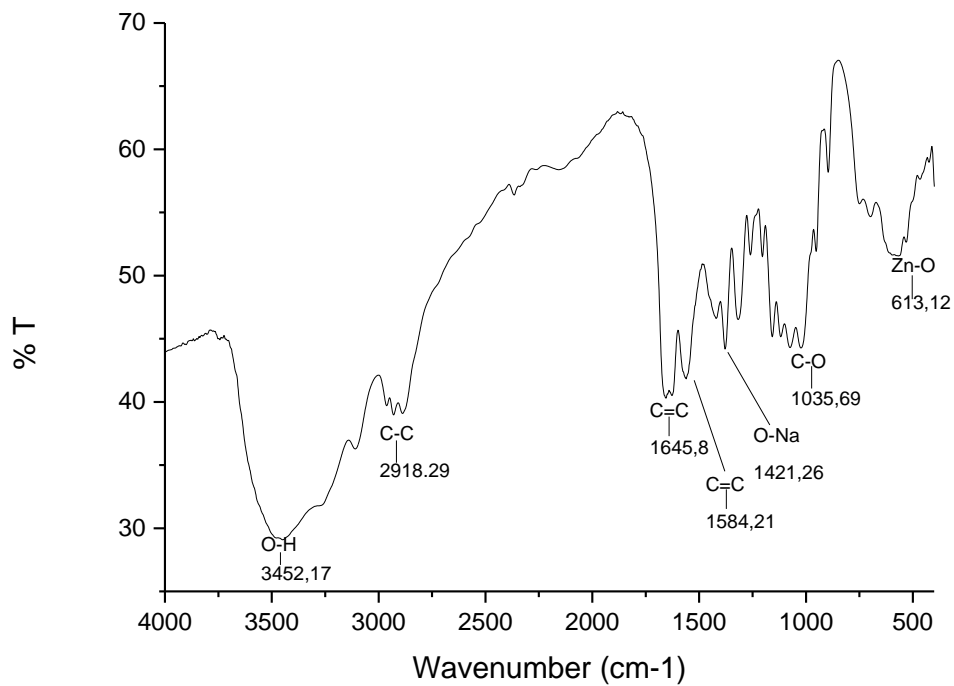


Figure 3. The spektrum FTIR sponge C (Comparison of curcumin:chitosan 1:4)

Absorb Capability

The ability to absorb sponge shows the amount of fluid that can be absorbed in the sponge is calculated using the formula:

$$E = \frac{W_e - W_o}{W_o} \times 100\%$$

We show the weight sponge that has absorbed PBS and W_o is the original weight. Three repetitions are done and the average of the repetition is used. The texture of the absorbent dressing sponge can be seen in Figure 4 and the capability of absorbing is shown in Figure 5.

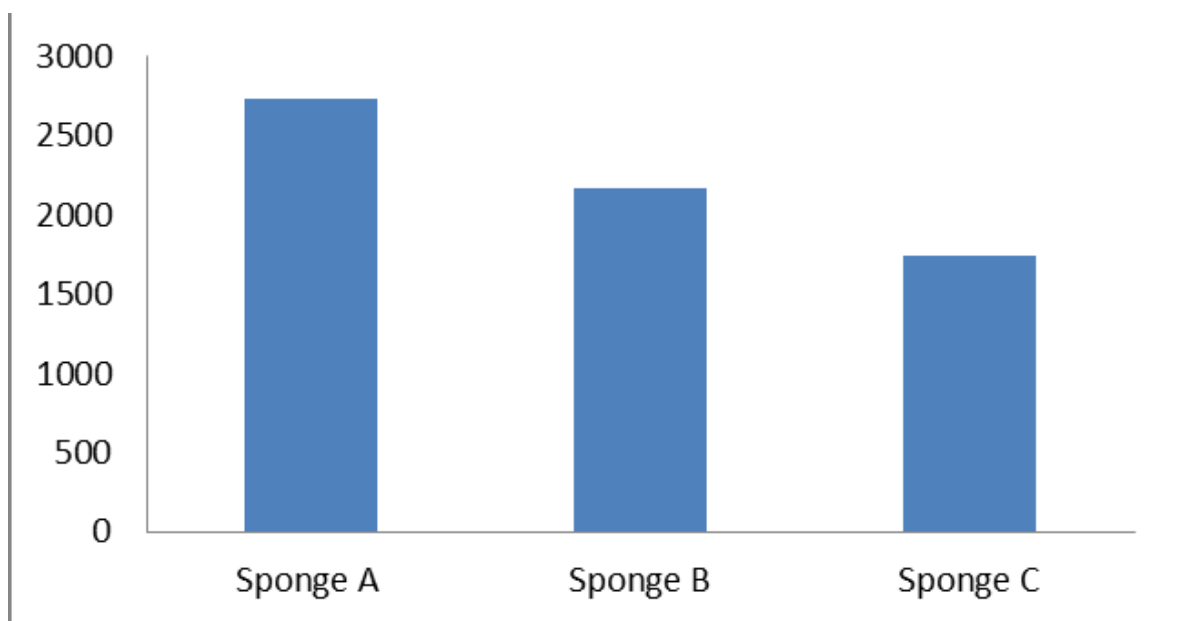


Figure 4. The Texture of Absorbent Dressing Sponge

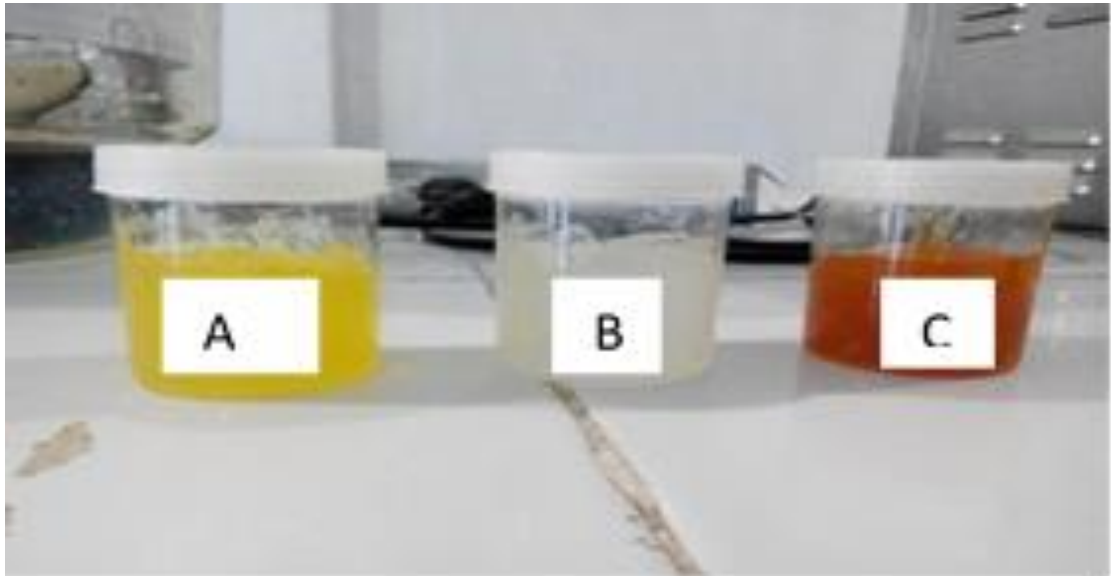


Figure 5. The absorbent capability of sponges in percentage.

The Standard of water

To find out the amount of percentage of water content in the sponge, it is necessary to conduct a test on water standard. To calculate the percentage of moisture content, it uses the formula below:

$$\% \text{ water standard} = \frac{W_o - W}{W_o} \times 100 \%$$

W_o shows an initial weight of sponge and W indicates the final weight after heating. The percentage of water content can be seen in table 2.

Table 2. Percentage of Water Standard

Sponge	% Water Standard
A	32,7
B	22,3
C	45,5

Histopathology

The histopathological test is done with the previous anatomical perform treatment in rabbits. The rabbits are treated with an incision for 5 days, and after 5

days of rabbit skin slashed and made preparations, the structure can be seen in a microscope. The result of the test can be seen in figure 6, figure 7 and figure 8.

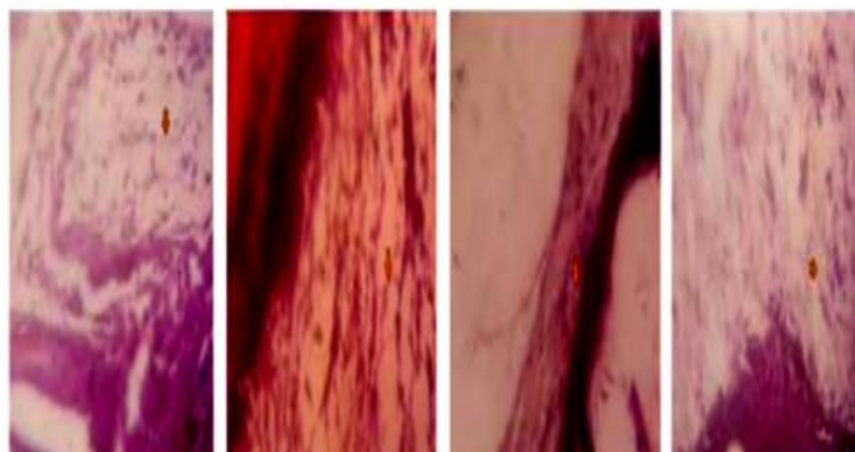


Figure 6. Re-epithelialization rabbit skin day 5.

The dotted lines indicate the injured area. Control (Mebo), sponge A (curcumin: chitosan 1:2), Sponge B (curcumin: chitosan 0:4), and Sponge B (curcumin: chitosan 1:4)

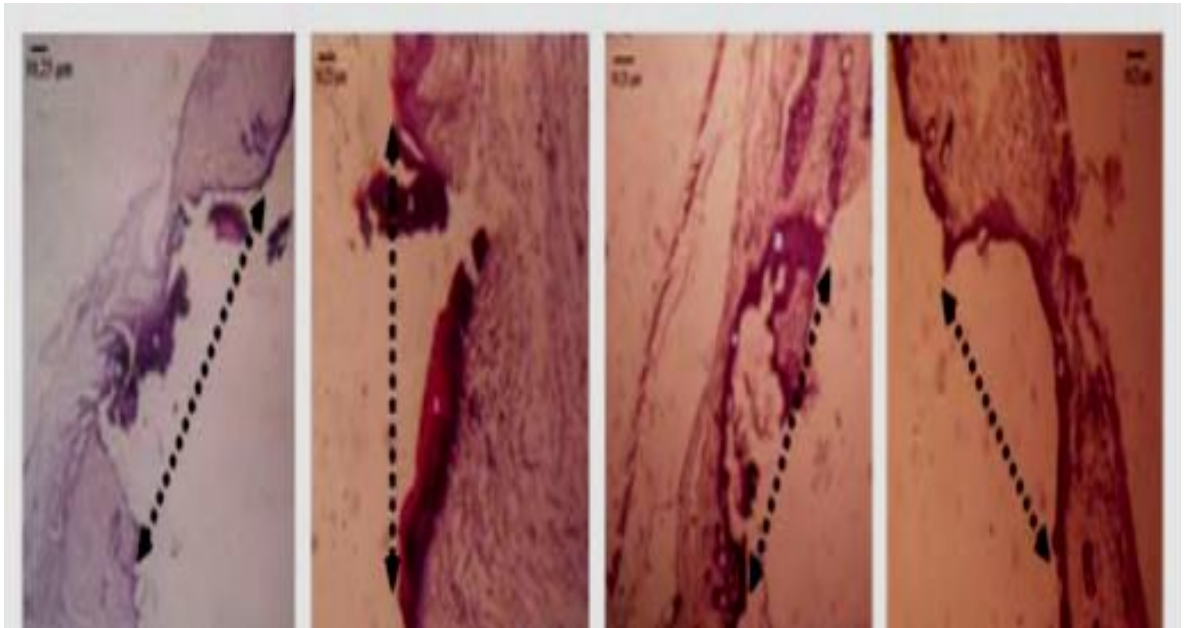


Figure 7. The collagen density (arrow) rabbit skin day 5 at 400X magnification. Control (Mebo), sponge A (curcumin: chitosan 1:2), Sponge B (curcumin: chitosan 0:4), and Sponge B (curcumin: chitosan 1:4)

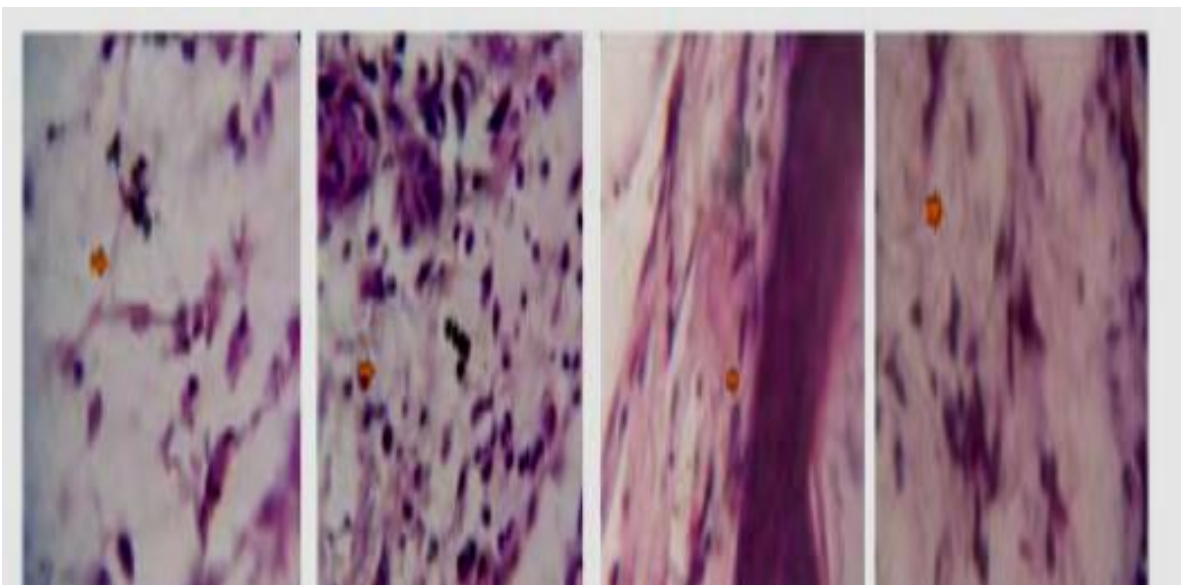


Figure 8. The density of collagen (arrows) rabbit skin day 5 at 400X magnification. Control (Mebo), sponge A (curcumin: chitosan 1:2), Sponge B (curcumin: chitosan 0:4), and Sponge B (curcumin: chitosan 1:4)

The parameters measured are % re-epithelialization and collagen density. Both of these parameters play an important role in wound healing. Re-epithelialization is the process of repair of the epithelial cells of the skin so that the wound will be closed. The faster the re-epithelialization will make structure skin epidermis and reach a normal state, while collagen is the major

protein components that make up the extracellular matrix and is the largest protein found in the human body. The percentage of re-epithelialization can be calculated using the following formula and the result of re-epithelialization can be displayed in table 3.

$$\text{Re-epithelia} = \frac{\text{the length of the wound is concealed by}}{\text{the length of total injury}} \times 100\%$$

Table 3. Percentage Re-epithelialization

Sponge	The Length of Injury (cm)		Re-epithelialization
	Total	Concealed by epithel	
Control	2,45	1,37	46,06%
A	2,52	0,32	87,3%
B	2,52	0,55	78,3%
C	2,57	1,57	100 %

Based on Table 3, the percentage of re-epithelialization of the rabbit skin that is given sponge is bigger than just given Mebo (control). The high percentage of re-epithelialization of wounds covered by sponge compared to the control is alleged because of the content of curcumin, chitosan, alginate, and Nano ZnOsponge. The curcumin is a phenolic group of secondary metabolites that

functions as an antibacterial that accelerates wound closure. The collagen density is calculated using the scoring parameters. The histopathological scoring parameters for collagen density was based on the measurement object of 1000x magnification [13]. The results of scoring collagen density between control and sponge can be seen in table 4.

Table 4. Collagen Density Assessment Score

Sample	Collagen Density Score
Control	+1
A	+3
B	+2
C	+4

Note:

- +0 = none of the collagen fibers in the injured area.
- +1 = density of collagen fibers in the injured area is low.
- +2 = density of collagen fibers in the injured area is medium.
- +3 = density of collagen fibers in the injured area is tight.
- +4 = density of collagen fibers in the injured area is very tight.

Cytotoxicity (MTT Assay)

The sponge cytotoxicity assay is performed on A, B, and C by using fibroblast cells. To calculate the percentage of living cells, it needs to use the following formula:

$$\text{Living cell} = \frac{OD \text{ treatment} + OD \text{ media control}}{OD \text{ Cell Control} + Media \text{ Control}} \times 100\%$$

If the percentage of living cells is less than 100%, then the material is said to be toxic [14]. The data percentage of a living cell are presented in Table 5.

Table 5. Percentage of Living Cells

Repetition	Living Cell (%)
Sponge A	107
Sponge B	101
Sponge C	119

Based on Table 5, the percentage of living cells of the three sponges magnitude is above 100%. It shows that alginate, chitosan, curcumin, and nano ZnO are truly used as raw materials for making absorbent dressing sponge is safe to use on skin, because it is biocompatible with fibroblasts.

Animal study

The test of effectiveness on healing wound was done on three rabbits that were divided into four groups treatment, they were: controlling group (Mebo), sponge A (curcumin: chitosan 1:2), Sponge B (curcumin: chitosan 0:4) and Sponge C (curcumin: chitosan 1:4). Hisphatology anatomy test was done after the rabbit's hair on the backside was cut. Then, it was divided into three parts and created an incision wound. The parameter observed was the percentage of re-epithelization and the collagen solidity. These parameters took a significant role in the healing process.

Statistical analysis

The statistical analysis was done by using Statistical Product and Service Solution (SPSS) verse 17.00. This analysis was conducted to observe the difference that

probably happens of the three groups experiment toward the healing process of rabbits' wound. The result showed that the decreasing wound diameter could be the same as the positive control treatment. Besides, the positive control treatment used was MEBO preparation containing three extracts, including *Radix Scutellariae*, *Cortex Phellodendri*, and *Rhizome Coptidis*.

1. Results and Discussion

In Sponge A, group C=C aromatic that becomes a part of curcumin appears on the ribbon catchment area on 1584.21cm⁻¹. The carbonyl group absorption band is a group of curcumin alginate and chitosan can be seen at ratio number of 1645.78 cm⁻¹. The Hydroxyl group (OH) is available on the three ingredients are alginate, chitosan, and curcumin. The group absorption band is found in the value of the ratio number of 3452.17 cm⁻¹. The CO group absorption band and O-Na are in the ratio number of 1035.69 cm⁻¹ and 1421.28 cm⁻¹, while the primary amide groups (NH₂) is concealed by a hydroxy group-wide ribbon for their hydrogen bonds between molecules.

On the data of FTIR spectra Sponge B, the absorption band of group C-C aliphatic can be seen at ratio number of 2918.29 cm⁻¹. The Hydroxyl group (OH) that becomes a group of chitosan appears on the value of the ratio number of 3427,85cm⁻¹, while the absorption band NH₂ group is concealed by a widening hydroxy group absorption band due to the hydrogen bonds between molecules.

On the other hand, on the data of FTIR spectra Sponge C, an absorption band of the carbonyl group that becomes a group of curcumin and alginate appears in the catchment area of 1627.63 cm⁻¹. The CO group absorption band and O-Na appear at ratio number of 1028.84 cm⁻¹ and 1404.89 cm⁻¹. The Cluster hydroxy (OH) appears on the ribbon catchment area of 3452.17 cm⁻¹. The Hydroxy group is a group that is available at the alginate, chitosan, and curcumin, while the absorption band NH₂ group is

concealed by a hydroxy group absorption band widening due to the hydrogen bonds between molecules.

Based on the test results of FT-IR shows that the four ingredients (alginate, chitosan, curcumin, nano ZnO) are mixed in the sponge. It can be seen from the emergence of functional groups of alginate, chitosan, curcumin, and Nano ZnO in the sponge. The carboxyl functional group of alginates which appears in the range of ratio number of 1765-1645 cm^{-1} , the OH in the range of 3650-3200 cm^{-1} , the CO in the range of 1260-970 cm^{-1} , and the O-Na at

From the measurement results with PSA (Particle Size Analyzer) based on the intensity, number and size of the acquired volume average amount to 154.07 nm Nano ZnO. It can be said that zinc nanoparticles synthesized as following the definition described by Mohanraj and Chen are solid particles with a size of 10-1000 nm [15].

Based on the five tests, it can be stated that among the three sponges that have good absorb (sponge A, sponge B, and sponge C) with a ratio of alginate: chitosan 1:4, have great re-epithelialization percentage and collagen density. As an absorbent dressing (except for absorption) should also pay attention to the healing process, because its purpose as a wound dressing is to accelerate wound healing. Besides, sponge C also shows the non-toxic nature and high water standard. Therefore, it creates a moist wound environment (moist wound healing) that it runs good oxygenation process.

Based on the test results of absorbing ability, there obtained 3 sponges that own absorb capability and good texture after tested with an emulsion of PBS pH 7.4. The three sponges are namely A, B, and C with a ratio of curcumin: chitosan 1: 2; 0: 4; 1: 4 that each absorbs a percentage of 2737%, 2167%, and 1745%. The three samples pick the chitosan composition ratio greater than curcumin. The rapid process of absorbing sponge is 35 seconds when it absorbs fluid PBS (Phosphate Buffer Saline), because the sponge forms a polyelectrolyte complex as alginates that are polyanions (negatively charged) and that the chitosan is Polikation (positively charged) accelerate the absorption of fluids, because the ionic of alginate and chitosan have a great potential to attract water molecules by forming hydrogen bonds [16].

In this study, the absorbent dressings for sponge A, B, and C were made with a ratio of curcumin and chitosan equal to 1: 2; 0: 4; 1: 4 to test the effect of wound healing. The healing passes through a series wound healing process consisting of 3 phases, such as the inflammatory phase, the proliferation phase (fibroblastic phase) and exclusion (maturation phase). The inflammatory phase was characterized by swelling and formatting bullae. Then, the proliferation phase occurs in the collagen and is characterized by the release of scab and the formation of reddish granulation tissue. The process of termination or maturation is marked by the closure of the wound as a whole by the new tissue with 0 cm diameter of burns. The main ingredients, such as sodium alginate, chitosan, curcumin, and ZnO nano serves to inhibit the growth of germs, microbes, fungi, bacteria and to accelerate wound healing.

approximately 1431 cm^{-1} , while the typical absorption group of chitosan is hydroxy (OH) and the primary amine (NH₂). The OH appears in the range of ratio number of 3650-3200 cm^{-1} absorption band NH₂ group that is concealed by a widening absorption band hydroxy group because of their hydrogen bonds between molecules. So, the curcumin has distinctive clusters that are not available at the alginate and chitosan. Those clusters are aromatic CH and C=C aromatic.

Based on several effectiveness tests, including absorbency test, water level test, cytotoxicity test, Anatomical Histopathology Test (HPA) carried out in this study, it can be stated that between three sponges (sponge A, sponge B, sponge C), sponge C which has a good effect on wound healing, which has good absorbency with a value of absorbing percentage rises to 2737%. Sponge C has greater a ratio of chitosan composition than curcumin. The speed of the absorb sponge process (35 seconds) when absorbing liquid Phosphate Buffer Saline (PBS) is caused by sponges forming complex polyelectrolytes because alginate and chitosan accelerate fluid absorption. It happens since the ionic side of alginate and chitosan has great potential to attract water molecules by forming hydrogen bonds [10]. As an absorbent dressing, aside from being absorbed, it must also pay attention to the healing process because the purpose of wound dressing is to accelerate wound healing.

Sponge C which has a ratio of curcumin: chitosan 1:4 has the highest water content in the percentage of 45.5%. The high levels of water in the sponge which has a ratio of chitosan is greater than curcumin is caused by hydrogen that bonds between chitosan and water that are used as a medium for dilution of acetic acid. Chitosan has the capability of binding water as it is supported by the presence of polar groups (CO) and non-polar (CH and CC) on chitosan. According to its chemical formula, chitosan has more than Curcumin OH groups, so that the ability to form hydrogen bonds with water molecules is greater. This hydrogen bonding causes the water content in the sponge is still high despite being steamed with lyophilizer process.

Sponge C also shows non-toxic properties with 119% value. This shows that alginate, chitosan, curcumin, and ZnO nano are safe to use on the skin because it is biocompatible with fibroblast cells and high moisture content with a percentage of 45.5%. Additionally, it creates a moist wound healing environment so that the oxygenation process runs well. Gas permeability in bandages is an important factor in wound closure where the exchange of oxygen and carbon dioxide has an effect on oxygen concentration in the wound tissue ultimately affects cellular wound healing. The high water content in sponge C whose chitosan ration is greater than curcumin due to the hydrogen bond occurring between chitosan and water used as a medium for dilution of acetic acid. Chitosan can bind water, this is supported by the presence of polar (C-O) and non-polar groups (C-H and C-C) on chitosan.

Under its chemical formula, chitosan has more O-H groups than curcumin so the ability to form hydrogen

bonds with water molecules is greater. It causes the water content in the sponge keeps being high even though it has been applied with the lyophilizer process. Besides Sponge C with a ratio of alginate: chitosan 1: 4 with the anatomical histopathological test, the parameters observed were re-epithelialization and collagen percentage. These two parameters play an important role in wound healing. Re-epithelialization is the process of repairing skin epithelial cells which can close the wound. The faster re-epithelialization, the more the epidermal structure and skin reach a normal state. Collagen is the main protein composing the extracellular matrix component and has much protein which is good for the body. The interaction between curcumin and collagen will form hydrogen bonds that can increase the density of collagen.

Conclusion

The result of FTIR test on alginate chitosan curcumin hydrogel and ZnO nano had formed a sponge through the occurrence of carbonyl absorbent tape, C-O and O-Na made from alginate, hydroxyl clusters (O-H) and premiere anima (NH₂) made from chitosan, C-H clusters aromatic and C=C aromatic from curcumin. Further, the existence of ZnO nano on the waving calculation equals to 613.12 cm⁻¹. Moreover, the result of PSA measurement which was based on intensity, numbers, and volume showed that the average size of ZnO nano was 154.07 nm. Also, it produced sponge C whose chitosan and curcumin comparison equals to 1:4 that has good texture and absorbent capability, 45.5% water and 100% of its re-epithelization. After it was tested with PBS pH 7.4 essence, the absorbent capability of sponge C raised to 2737% and had no toxic with above 100% of living cells.

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Conflict of interest

This study has no conflict of interest.

Abstract

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This study carried out the test of the effectiveness of Hydrogel formula of turmeric extract - chitosan and ZnO nano for wound in *Oryctolagus cuniculus*, New Zealand rabbits, using Sodium alginate as a hydrogel agent. It was done to obtain a physically stable formula made from turmeric - chitosan and ZnO nano Hydrogel extracts to heal a wound. The stable hydrogels were tested further in vivo with three New Zealand rabbits and divided into three treatment groups, are stable Hydrogel preparations, positive controls, and negative controls. The results showed that stable hydrogel of chitosan turmeric extract and ZnO nano were effective to heal the wound as marked by a reduction in wound diameter faster than wound

diameter in negative control rabbits. Further in the Anova test with a Complete Randomized Design (CRD) data obtained F count value of 596 > F table 1% 10.92. Indeed, there is a very significant difference between the three treatment groups. Additionally, the result of Tukey's HSD (honestly significant difference) test obtained that from the three treatment groups has a significantly different effect with negative control and not significantly different from positive control; preparations that have been known to have a healing effect on wound.

Keywords: Absorbent Dressing, Alginate, Chitosan, Curcumin, ZnO Nano, Healing.

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