The synthesis and characterization of hydrogel chitosan-alginate with the addition of plasticizer lauric acid for wound dressing application

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Abstract. The writers conducted a study about the synthesis and characterization of hydrogel chitosan-alginate by addition plasticizer lauric acid for wound dressing application. The purpose was to find out the impact of lauric acid concentration variation on hydrogel chitosan-alginate to get the best mechanical and physical properties to be applied as wound dressing in accordance with existing standards. This study used commercially chitosan from extract of shells crab, commercially-available alginate from the extract of sargassum sp, and commercial lauric acid from palm starch. The addition of lauric acid was aimed to repair mechanical properties of hydrogel. The composition of chitosan-alginate is 4:1 (v/v), while the lauric acid concentration variations are 0%, 1%, 2%, 3%, 4%, and 5% w/v. The characterization of mechanical properties test (Tensile strength and Elongation at break) at hydrogel showed the hydrogel chitosan-alginate-lauric acid have the characteristic which meets the standard of mechanical properties for human skin. The best performance of hydrogel chitosan-alginate-lauric acid was obtained by increasing luric acid concentration by 4%, which has a thickness value of 125.46±0.63 µm, elongation 28.89±1.01 %, tensile strength (9.01±0.65) MPa, and ability to absorb liquids (601.45 ±1.24) %.

1. Introduction
Wound healing and tissue restoration is defined as a reparative process which usually consists of a series of continuous inflammation and repair. During this process, epithelial cells, endothelial cells, inflammatory cells, platelets, and fibroblasts interact to restore normal functions. Recently, a lot of research is conducted to find a method of regeneration and wound healing through the use of various cover materials (dressing) to facilitate proper wound management [1]. The research on wound closure has focused on accelerating wound repair by improving the design of the cover material. One type of wound closure material that is being developed is hydrogel. Hydrogels have the advantage to work well in a moist environment. This material is a natural absorbent of biodegradables through decomposition by microbes [2]. The use of a blend of two or more substances will provide hydrogel with good mechanical properties. The use of biological materials such as chitin and chitosan derivatives, of which itself has a fairly medical extensive uses [3]. The use of chitosan in wound management has many advantages due to its biocompatibility and molecular biodegradability that do not harm the environment. When chitosan is used as wound covering, besides biocompatible chitosan will also be biodegradable by lysozyme, chitinase, and kitosanase into oligomers and monomers (sugar amines), making it harmless and completely absorbable by the body [1]. Chitosan is a hemostat
that assist unnatural blood clotting, but this material has brittle and can be easily torn. The modification of the materials for better characteristics can be done by adding alginate. Alginate and chitosan are polyanionic and polycationic when dissolved under the right conditions and can interact with each other via the carbonyl group of the amino group of the alginate and chitosan. Alginate also has the advantage of being non-toxic, biodegradable, biocompatible, and does not cause allergies.

One previous study identified chitosan-alginate membrane potential as a candidate for hemodialysis membrane with the composition of 4:1 [4]. In the study, the elongation values obtained were not included in the standard range of mechanical properties of human skin. Increasing the elongation of the composite can be done by the addition of plasticizers. Plasticizer has a function to increase the flexibility and extensibility of the gel, avoid the gel from cracking, and improve the elasticity of the gel to increase the elongation of the hydrogel. One material that could potentially improve the mechanical properties of the hydrogel dressings is lauric acid [5]. Lauric acid is a medium chain saturated fatty acids found to be antimicrobial (antiviral, antibacterial, and antifungal). Lauric acid in the body and on the skin will turn into monolaurin, which can kill bacteria and thus speed up the metabolism of cells in the skin. This created a base for the present study on the synthesis and characterization of chitosan-alginate hydrogel with the addition of lauric acid plasticizer for the application of wound dressings.

2. Experiment design
In this study, the materials used are as follows: chitosan, Na-alginate, lauric acid, glacial acetic acid, distilled water, PBS, KBr, and 96% ethanol. The tools used are glass beaker, spatulas, paper weights, pipettes, stirrers, aluminum foil, measuring cups, glass mold, digital balance, and magnetic stirrer. Equipment for analysis of quantitative and qualitative Coating Thickness Gauge is a type TT 210, Bruker Tensor 27 FTIR and IMADA Tensile machine type HV-1000N.

The materials are chitosan-alginate prepared in a ratio of 4:1 with the addition of lauric acid (plasticizer) solution of 0% w/v, 1% w/v, 2% w/v, 3% w/v, and 5% w/v. The preparation of alginate required 3 grams of alginate powder that was mixed with 100 ml of distilled water. Furthermore, the stirring was conducted at room temperature until the solution became homogeneous. Alginate was added slowly while stirring occurs. The chitosan solution required 2 ml of glacial acetic acid. They were mixed with 3 grams of chitosan powder ready to use along with 98 ml of distilled water, which were then stirred for 45 minutes. A 10 ml alginate solution was then added gradually into a solution of 40 ml chitosan which was still in a state of stirring. The solution was then added with lauric acid (plasticizer) with a composition variation of 0% w/v, 1% w/v, 2% w/v, 3% w/v, and 5% w/v which had been melted at a temperature of 44 °C, which is the melting point of lauric acid Lauric acid is a saturated fatty acid that is required to dissolve 96% ethanol. After all the ingredients were mixed, stirring was continued for 30 minutes in order to obtain a homogeneous solution.

The hydrogel solution that has formed was poured and leveled on a glass plate with a thickness of 2-4 mm. Then, the solution that had been leveled at the glass plate was dried at room temperature for 7 days until the hydrogel was completely formed in sterile condition.

2.1. Thickness test
The thickness test of the test sample was performed using the Coating Thickness Gauge TT type 210. The Coating Thickness Gauge type TT 210 was put above the hydrogel samples in order to obtain the data. These measurements aimed to determine the effect of variations in the composition of the chitosan-alginate with lauric acid addition to the thickness of the particular test sample produced.

2.2. Hydrogel tensile strength test
The mechanical characteristics observed from a chitosan-alginate hydrogel material with the addition of lauric acid plasticizer were measured with a tensile test using an IMADA type HV-1000N tensile machine. The tensile test was conducted to determine the mechanical properties such as strength,
elasticity, rigidity, and plasticity. The determination of mechanical properties was done by cutting the hydrogels of the appropriate size. The ends of hydrogels were linked to test equipment and towing load mounted on the unit load N. The hydrogel was drawn at a certain speed to cause drop. The maximum towing load (fmax) and the change in length hydrogels at break was recorded. The results obtained by the value of the stress and elongation at break were calculated using the equation [7]:

$$\sigma = \frac{F}{A}$$

with \( \sigma \) = Stress (N / m²), \( F \) = Load (N), \( A \) = surface area (m²).

$$\varepsilon = \frac{L - L_0}{L_0} \times 100\%$$

with \( \varepsilon \) = elongation (%), \( L \) = length of the end of the test specimen (cm), \( L_0 \) = initial length of the test specimen (cm).

Hydrogel of chitosan, alginate and lauric acid as plasticizer can be used as a wound cover (Wound Dressing) if it meets the standard of certain mechanical properties.

2.3. Absorption ability test
The absorption capacity of the hydrogel was determined by incubating the hydrogel at pH 7.4 in Phosphate Buffer Saline (PBS) at room temperature. Hydrogel wet weight was calculated for several times to let sponge filter paper remove the water adsorbed on the surface and then immediately weighed with digital scales. The amount of water absorbed by the hydrogels can be calculated using the equation:

$$E = \frac{m_e - m_o}{m_o} \times 100\%$$

where \( E \) is the percentage of absorption of water in the hydrogel. \( m_e \) stands for the weight of hydrogel that had absorbed PBS, while \( m_o \) is the initial weight.

2.4. Fourier Transform Infra Red (FT-IR) Test
The FT-IR spectroscopy test was conducted by eroding the chitosan-alginate hydrogel-lauric acid together KBr with a ratio of 1:10 (w / w). The mixture was then pressed by using presses at a pressure of 10 tons, so that the pellet became solid. These pellets are then analyzed using Bruker Tensor spectroscopy type 27. The results of FT-IR spectra was obtained in the form of illustrations of the %value of transmittance and wave number, so it can be any functional group contained in chitosan-alginate hydrogel-lauric acid.

3. Results and discussion

3.1. Fourier transform infra red (FT-IR) test
Figure 1. The IR spectrum-Chitosan Hydrogel with Alginate Lauric Acid Addition

| Functional Group | O-H (Stretching) | O-H | C=C | N-H (Bending) | C=O (Stretching) | C=O | C-H (Stretching) | C-H (Bending) | N=O=O |
|------------------|------------------|-----|-----|---------------|------------------|-----|-----------------|---------------|-------|
|                   | 3291.10          | 2870.83 | 2356.73 | 1647.73       | 1419.94          | -   | 1088.59         | 1405.70       | -     |
|                   | 3221.74          | -    | -    | -             | -                | -   | 1089.69         | 1448.64       | -     |
|                   | 3319.98          | -    | -    | 1557.12       | -                | -   | 1088.59         | -             | -     |
|                   | 3249.78          | -    | -    | 1539.13       | 1705.80          | -   | 1064.91         | -             | -     |
|                   | 3259.75          | -    | -    | 1538.36       | 1603.17          | -   | 1064.91         | -             | -     |
|                   | 3252.99          | -    | -    | 1538.19       | 1596.83          | -   | 1064.80         | -             | -     |
|                   | 3245.05          | -    | -    | 1541.34       | 1633.84          | -   | 1065.37         | -             | -     |
|                   | 3272.40          | -    | -    | 1538.97       | 1632.71          | -   | 1065.80         | -             | -     |
|                   | 3258.09          | -    | -    | 1538.19       | 1635.19          | -   | 1072.34         | -             | -     |

Table 1. Data of absorption spectrum

The shift in the wave number of functional groups was due to an increase or decrease in the radiation energy absorbed by the molecules. In chitosan-alginate hydrogel with the addition of 4% lauric acid, there was no C-H groups, but there was a new functional group, namely N = C = O at wavenumbers of 2356.73 cm⁻¹ and 2334.56 cm⁻¹. The new functional group was formed by the reaction between the primary amine group (NH₂) on chitosan and carboxyl group (COO) on alginate and lauric acid. In addition, the formation of two peaks was due to their symmetrical and asymmetrical vibration. The interaction between the primary amine group with a carboxyl group formed an amide bond (-NH-C = O). The amide bond underwent rearrangement by shifting electrons forming a new functional group (-N = C = O) that they can be turned into electron structures of (N CO-), (-N = C = O + R +), (-N + CO⁻), and (-N = C = O). Thus, the crosslink forming reaction between chitosan-alginate by the addition of lauric acid took place more quickly so that the resulting structure became more dense.

The chitosan-alginate hydrogel with the addition of 4% lauric acid is the chemical compound with the advent of a new functional group so that the hydrogel has a chemical structure that is much different from their constituent components. A greater concentration of the resulting bond of the new group made the molecules of the polymer blend to be denser. This meeting of molecules may cause effects on the macroscopic properties of the hydrogel.
Table 2. Characterization of chitosan-alginate hydrogel-lauric acid

| Sample (%) | Thickness (μm) | Elongation (%) | Tensile Strength (MPa) | Absorption Percentage (%) |
|------------|----------------|----------------|------------------------|---------------------------|
| 0          | 80.96 ± 2.54   | 6.90 ± 0.89    | 27.91 ± 1.55           | 400.98 ± 1.50             |
| 1          | 92.66 ± 1.22   | 11.11 ± 0.93   | 10.81 ± 1.18           | 482.85 ± 1.45             |
| 2          | 103.76 ± 0.95  | 13.66 ± 0.94   | 9.87 ± 0.94            | 485.59 ± 1.34             |
| 3          | 115.46 ± 0.64  | 17.78 ± 0.96   | 9.51 ± 0.76            | 529.71 ± 0.93             |
| 4          | 125.46 ± 0.63  | 28.89 ± 1.01   | 9.01 ± 0.65            | 601.45 ± 1.24             |
| 5          | 128.46 ± 0.29  | 16.29 ± 0.95   | 10.18 ± 0.62           | 340.12 ± 0.91             |

3.2. **Thickness test**
Based on Table 2 description of chitosan-alginate hydrogel-lauric acid values of different thickness, it can be explained that along with the increase in the addition of plasticizer concentration of lauric acid, the thickness of the hydrogel has a tendency to increase. The more of lauric acid is added in the hydrogel, the more water is trapped in the starch granules because lauric acid inhibits the evaporation of water during the drying so that the resulting hydrogel gets thicker. Hydrogel can be used as wound coverings based on this finding.

3.3. **Hydrogel tensile strength test**
The elongation values of hydrogel showed that with increasing concentration of lauric acid, hydrogels’ elongation values also increase. At a concentration of 5%, elongation values declined, but the value is still above the value of the elongation hydrogels without the addition of lauric acid. In Riki Siswanto (2013), chitosan-alginate membrane consisting of a mixture of chitosan and alginate and without the addition of lauric acid plasticizer produced small elongation values in the range 3.1% - 13.1%. This proves the effect of the addition of lauric acid which serves to increase the value of elongation hydrogel. The amount of elongation determines ductility of a material, and if its value is close to zero, the material is a brittle material [6]. Increasing elongation value resulted in more elastic hydrogel when applied to the skin surface. Increased elongation of chitosan-alginate hydrogel-lauric acid occurs because the plasticizer molecules of lauric acid has a strong enough force interaction with polymer hydrogels that plasticizer molecules diffuse into the polymer chain (between the polymer chitosan and alginate). The addition of lauric acid plasticizer of more than 4% shows the results of the elongation decreased. This occurred because the addition of lauric acid was exceedingly saturated so excessive plasticizer molecules are in a separate phase outside the phase of the hydrogel. The situation causes a decrease in the intermolecular force between the chains.

Values for tensile strength is inversely proportional to the value of elongation, which is more and more increasing concentration of lauric acid, then the value of tensile strength of the hydrogel decreases. However, at concentrations of 5% tensile strength values increase again. This is in line with the value of elongation at 5% concentration. The tensile strength of a material is influenced by its constituent chemical bonds. According to Park et al. [7], the shape, the number of carbon atoms in the chain, and the number of hydroxyl groups contained in the plasticizer molecules will affect the mechanical properties (tensile strength and elongation) of a hydrogel. Plasticizer will decrease the hydrogen bonds within the hydrogel to increase the flexibility of the hydrogel where the increase flexibility of the tensile strength of the hydrogel will be smaller [8].

Chitosan-alginate hydrogel-lauric acid can be used as a medical material if it meets a certain standard of mechanical properties as shown in Table 1. Based on the research Jansen and Rottier
(1958), that threshold for medical materialists an elongation value between 17% - 207% and the value of tensile strength of between 1 MPa - 24 MPa. From the data analysis, then the chitosan-alginate hydrogel-lauric acid with a composition ratio of 4: 1: 3 and 4: 1: 4 (v / v) was selected as the best sample because it has a value of elongation of (17.78 ± 0.96)% and (28.89 ± 1.01)% with a value of tensile strength of (9.51 ± 0.76) MPa and (9.01 ± 0.65) MPa, fitting in the range as a medical material wound closure proposed by Jansen and Rottier (1958), wherein the wound closure can be applied to human abdomen.

3.4. Absorption ability test
Hydrogel dressings formed from the ring insoluble polymers. It has a high water content which allows this material to be ideal as cover for wounds [9]. Hydrogel does not dissolve in water because it only not only absorbs and releases water, but also provides nutrients proportionately when needed. Hydrogels can absorb water as much as 1000 times the weight of the hydrogel itself.

The addition of lauric acid in chitosan-alginate hydrogel made solution of PBS (Phosphate Buffer Saline) absorbed to increase, but the addition of lauric acid more than 4% of the hydrogel decrease its ability to absorb. This happens because of the addition of lauric acid that passes through the saturation limit causes excessive plasticizer molecules that are in a separate phase from hydrogel. Hydrophobic properties contained lauric acid result in a less efficiency, making it not compatible. This is supported by the results of tensile strength tests (tensile strength) which indicates the effective value of elongation of hydrogel with the addition of lauric acid is not more than 4% (w / v).

4. Conclusion
Based on the results obtained in this study, it can be concluded that:
1. Variations in the composition of the lauric acid plasticizer in chitosan-alginate hydrogel yields effect to the mechanical and physical properties of the material. The higher the addition of lauric acid plasticizer, the higher the thickness, elongation, and the ability to absorb the chitosan-alginate hydrogel-lauric acid increased, while the value of tensile strength decreased.
2. The most suitable characteristics of chitosan-alginate hydrogel-lauric acid for standards to be applied as a wound dressing is shown by chitosan-alginate hydrogel with the addition of 4% lauric acid, wherein the hydrogel has a thickness of (125.46±0.63) μm, elongation of (28.89 ±1.01)%, tensile strength of (9.01± 0.65) MPa, and capability of absorbing fluid of (601.45±1.24) %.

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