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# Utilization of Broiler Skin Gelatin as Wound Healing Medicine

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**Abstract**

Gelatin is a protein-derived compound obtained by extracting animal collagen. Gelatin can be extracted from broiler chicken skin and used as a wound therapy drug. This study aims to explain the effect of using chicken skin gelatin on the healing process of cut wounds in animal models of mice. The 25% chicken skin gelatin ointment formulation was homogenized with ointment additives such as 0.2% preservative, 3.7% emulsifier, and 71% moisturizer. Testing the physical quality of spreadability and stickiness of chicken skin gelatin ointment using a T-test. Testing the ability to treat cuts using a Completely Randomised Design with 3 treatments namely P1 = administration of NaCl (negative control), P2 = administration of broiler skin gelatin ointment, and P3 = administration of Commercial ointment (positive control) each repeated 5 times. The test results of spreadability and stickiness of chicken skin gelatin ointment showed different results with commercial ointment. The chicken skin gelatin ointment produced is an ointment with a semisolid texture in the form of semi-stiff (high viscosity). The results of testing the treatment of cut wounds with three different treatments obtained results that were significantly different ($P < 0.01$) on the description of the level of wound healing, the percentage of wound healing, and histopathological observations. From the results of the research conducted, gelatin from broiler chicken skin is effective as a wound therapy drug and the healing process of cut wounds in mice is best in treatment using chicken skin gelatin ointment.

**Keywords:** Chicken skin, Collagen, Gelatin, Wound therapy drug.

**Full length article** *Corresponding Author, e-mail: irfanunhas@gmail.com; irfan.said@unhas.ac.id Doi # https://doi.org/10.62877/37-IJCBS-24-25-19-37

## 1. Introduction

Wounds occur due to cellular, anatomical, and functional disorders [1][2]. Wounds are experienced by every living thing. Wounds severely impact an individual's activities and cause weakness and joint cramps, so treatment is needed to accelerate the healing process. Treatment strategies rely on the application of topical medications formulated to promote wound healing, minimize the body's inflammatory response, and most importantly prevent opportunistic infections commonly associated with severe wound injury [3]. Therapies that can be carried out include the administration of topical ointments that have active substances according to the needs of damaged body tissues such as protein and collagen intake. Proteins such as collagen have promising characteristics for use as a base matrix for skin care products [4][5][6]. Gelatin is a protein obtained from the partial hydrolysis of collagen. Collagen is one of the proteins that make up the human body. Collagen has the function of giving skin strength and elasticity helping replace dead cells and accelerate healing of damaged cells. The abundant type of collagen is type I where this type of collagen is available in animal skin and is widely used in various medicines, one of which comes from chicken skin. Chicken skin contains high protein and collagen which are 73.12% and 87.21% [7]. Collagen is a fibrin protein that plays a role in maintaining shape and providing flexibility to tissues as well as an organic component of building bones, teeth, joints, muscles, and skin.

Gelatin was developed into a wound ointment because gelatin from chicken skin contains high collagen and in the gelatin process, there is a separation between saturated fat and collagen so that in the process of making gelatin ointments used purely contains chicken skin collagen which can maximize the healing process of wounds on the skin as well as an ideal biological matrix for ointment formulations [8]. Thus, the purpose of this study is to determine how the effect of chicken skin gelatin on the wound healing process, the utilization of livestock by-products, namely chicken skin so that it has functional value for the community, develop the utilization of collagen in chicken skin, and see the comparison of the treatment process using chicken skin gelatin with commercial treatment.

## 2. Materials and methods

### *2.1 Materials used*

The materials used in this study were broiler chicken skin from a slaughterhouse that already has halal standards (Bontoala Makassar), male mice aged 4 - 5 weeks from a mice farm (Gold Mice Farm Maros), sterile distilled water and 70% alcohol (PT. Hepilab Sukses Bersama Semarang), chloroform, formalin, NaCl 0.9% and Bioplasenton® (PT. Kimia Farma Indonesia), cera flava, propylparaben, metylparaben and pure white vaseline (CV. Dian Fajar Medika Yogyakarta).

### *2.2 Ethics approval*

The experimental protocol was approved by the Institutional Animal Ethics Committee, under the supervision of the Scientific Research Review Committee, Faculty of Veterinary Medicine, Hasanuddin University, Number UH23050300 on 16 June 2023.

### *2.3 Methods*

#### *2.3.1 Gelatin production process*

Cleaning 300 g of broiler chicken skin until there is no fat and dirt on the surface of the skin. Then extract using a waterbath at 60ºC for 24 hours. Filtering the broiler skin until the pulp is separated from the gelatin solution. The gelatine product is ready to be processed into ointment preparations.

#### *2.3.2 Ointment-making process*

Melt 28.310 g (71%) white vaseline and 1.490 g (3.7%) cera flava at 70ºC on a magnetic stirrer at 400 rpm until the temperature drops to 35ºC then mix 10 g (25%) gelatin, 0.008 g (0.02%) methylparaben, and 0.072 g (0.18%) propylparaben while continuously stirring the mixture until homogeneous and place in an ointment pot. The broiler skin gelatin ointment product is ready for application.

#### *2.3.3 Experimental test of gelatin ointment preparation*

Adapt the mice for 1 week so that they can adjust to the environment. During the adaptation process, mice are given food in the form of pellets and water ad libitum. Shave the mice fur with a diameter of 2 cm in the back area until it is slippery then clean it with 70% alcohol. Next, do the anesthetic route by inhalation using chloroform. Then make an incision wound with a length of 1 cm with a depth of 2 mm. Continue with drug administration with broiler skin gelatin ointment and commercial ointment by applying ointment to the mice wound. The ointment was applied twice a day at the same time repeatedly from day 1 to day 14 or until the wound was dry and closed.

#### *2.3.4 Observation parameters*

Testing ointment preparations directly based on [9][10] using two glass slides. Weighed 0.25 g ointment preparation, placed at the centre between two glass plates, Added 250 g weight placed in the middle of the plates. The time taken in seconds to separate the two slides was recorded to determine the spreadability of the ointment. The spreadability was calculated by the following equation:

$$S = \frac{M \times L}{T}$$

Where:
S : spreadability
M: weight placed on the top slide
L : Length of glass slide
T : time taken to separate the slides

Observations on the wound were made before the ointment application by taking pictures of the wound area using a digital camera with the same height distance and photo size. Taking pictures every day at the same time at 08.00 am before reapplication of ointment then measuring the wound using a tape measure while taking pictures of the wound using a digital camera. The purpose of testing the description of the wound healing rate is to determine the development of the wound every day after treatment. Then compare the results of wound development from 3 treatment treatments and measure changes in wound length using a measuring instrument with mm units after that calculate the percentage of healing rate with the following equation:

$$\frac{A - H\ (1, 2, 3, \ldots)}{A} \times 100\%$$

Where:
A: initial wound area
H: daily wound area

Histopathological observations were made on skin tissue preparations. Observations were made using a light microscope (Olympus SZ61) at 400x magnification using the scoring method. These observations included parameters that play a role in wound healing such as the formation of new blood vessels (neocapillarisation), growth in connective tissue (fibroblasts), and the presence of inflammatory cells (macrophages).

#### *2.3.5 Statistical analysis*

The data obtained were analyzed in a completely randomized design (CRD) with 3 trials and 5 replications using the GLM procedure of software [11]. The mathematical model was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:
Y : dependent variable
$\mu$ : overall mean
$T_i$ : effect of jenis pengobatan
$\varepsilon_{ij}$ : residual error

Duncan's test was used to determine the real difference between treatments. Differences with $P<0.05$ were considered statistically significant.

## 3. Results and Discussions

The spreadability of ointments can be categorized into three groups: low, medium, and high. In this study, the spreadability produced from chicken skin gelatin ointment was included in the medium group (Tables 1-3). Unlike the spreadability obtained from commercial ointments which are included in the high spreadability group. The spreadability of an ointment is determined by its texture and the ability to pull the attachment, the longer the attachment time, the lower the spreadability of the ointment and vice versa [10].

**Table 1.** Physical test values of the ointment used

| Type of ointment | The active ingredients used | T | Spreadability (g.cm/s) |
|---|---|---|---|
| Gelatin ointment | Broiler chicken skin gelatin | 14,02 | 109,23 |
| Bioplacenton® ointment | Placenta extract | 8,60 | 178,08 |

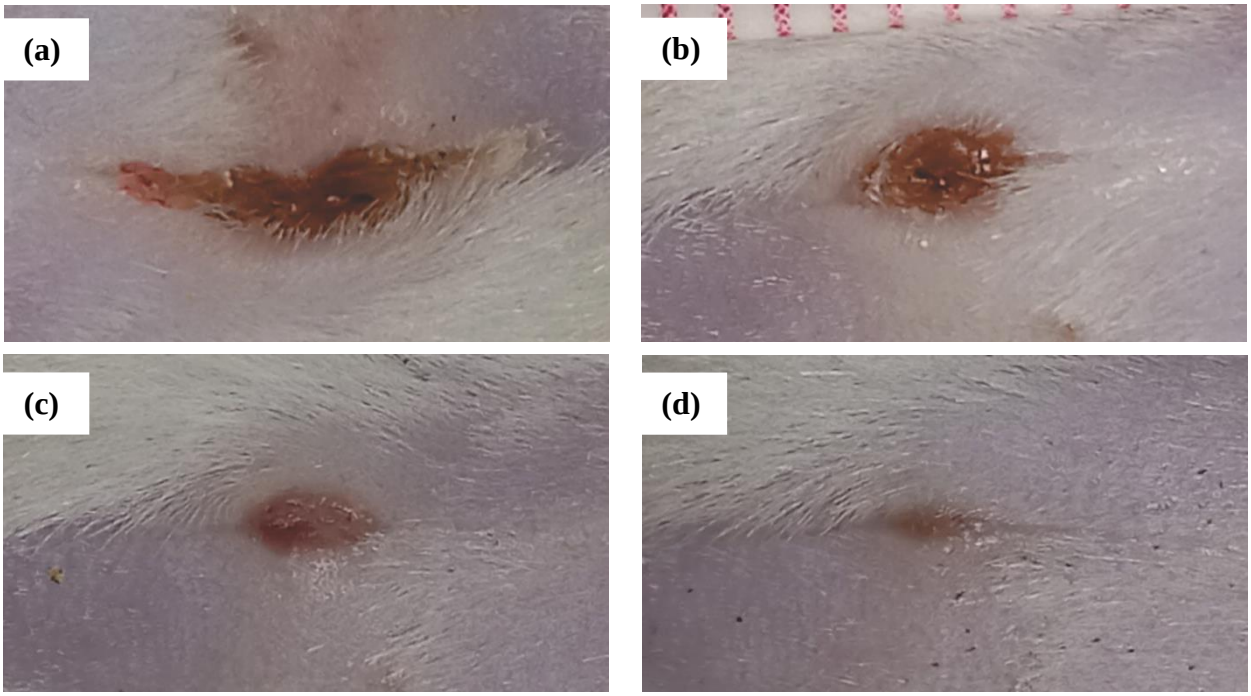



**Figure 1.** Overview of wound changes in mice treated with NaCl:
(a) Day 3, (b) Day 6, (c) Day 9, (d) Day 12.

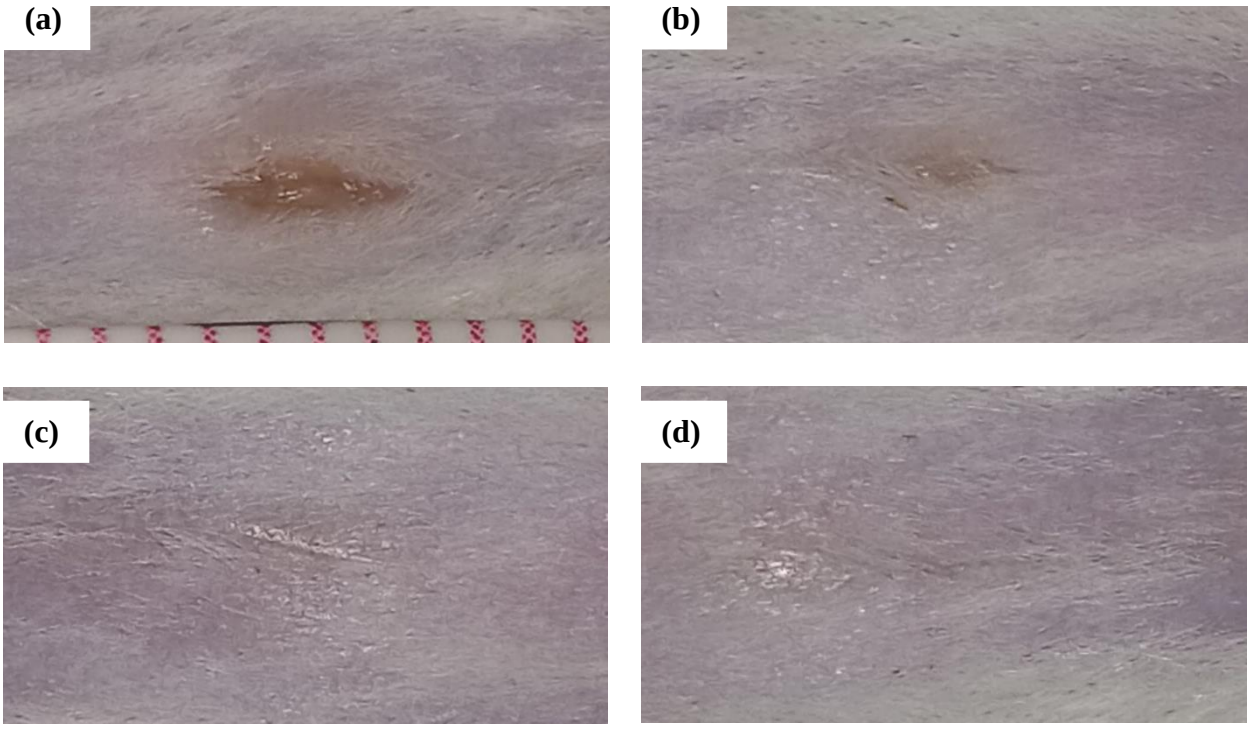



**Figure 2.** Overview of wound changes in mice treated with gellatin:
(a) Day 3, (b) Day 6, (c) Day 9, (d) Day 12.

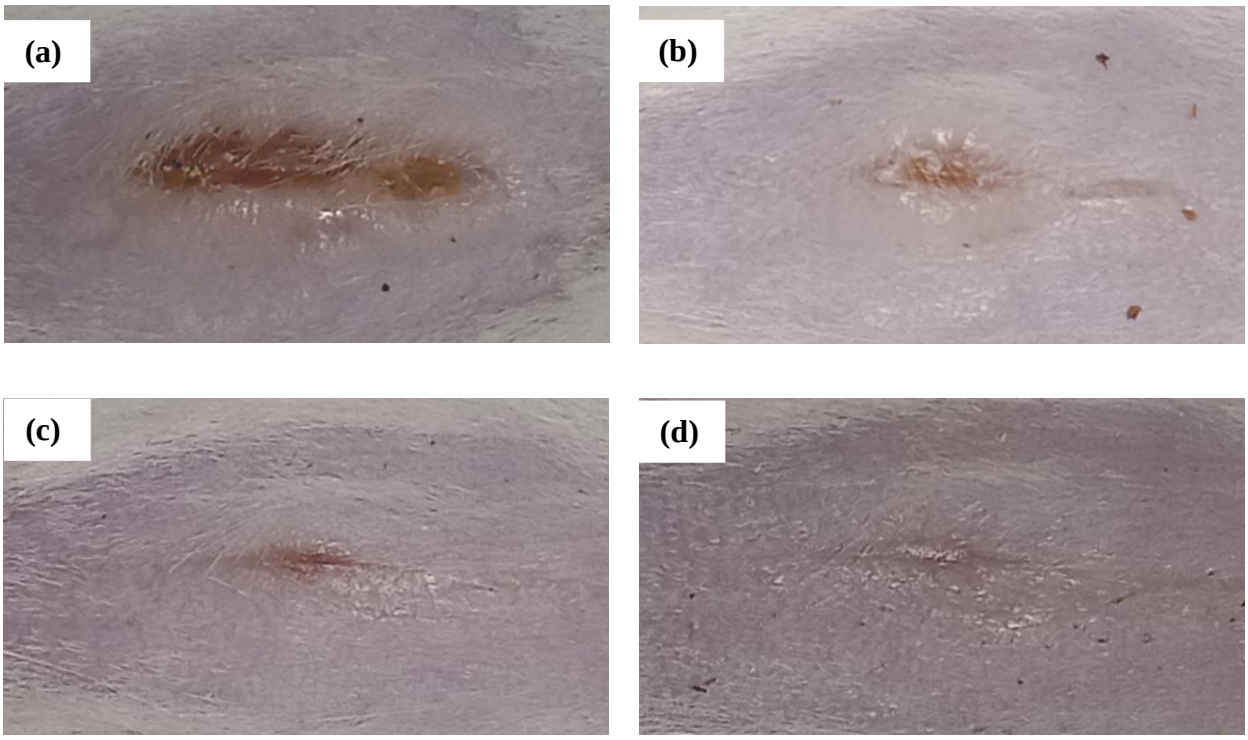



**Figure 3.** Overview of wound changes in mice treated with Bioplacenton®:
(a) Day 3, (b) Day 6, (c) Day 9,(d) Day 12.

**Table 2.** Percentage of wound healing rate (%)

| Day to | NaCl | Gellatin | Bioplacenton® | Average |
|---|---|---|---|---|
| 3 | 17,00±6,70 | 33,00±11,51 | 23,00±5,70 | 24,33±10,32[a] |
| 6 | 51,00±4,18 | 56,00±13,41 | 49,00±7,41 | 52,00±9,02[b] |
| 9 | 78,00±14,83 | 93,00±6,70 | 93,00±9,74 | 88,00±12,50[c] |
| 12 | 92,00±7,58 | 100,00±0,00 | 100,00±0,00 | 97,33±5,62d |
| Average | 59,50±30,56[a] | 70,50±29,37[b] | 66,25±33,12[b] | 65,41±30,86 |

[abcd]Different superscripts in the same row indicate highly significant differences (P<0.01).

**Table 3.** Results of Parameter Scoring on Mice Skin Preparations

| Treatment | Neocapillarisation | Fibroblasts | Macrophages |
|---|---|---|---|
| NaCl | ++ | + | +++ |
| Gellatin | +++ | +++ | ++ |
| Bioplacento® | ++++ | ++ | ++ |

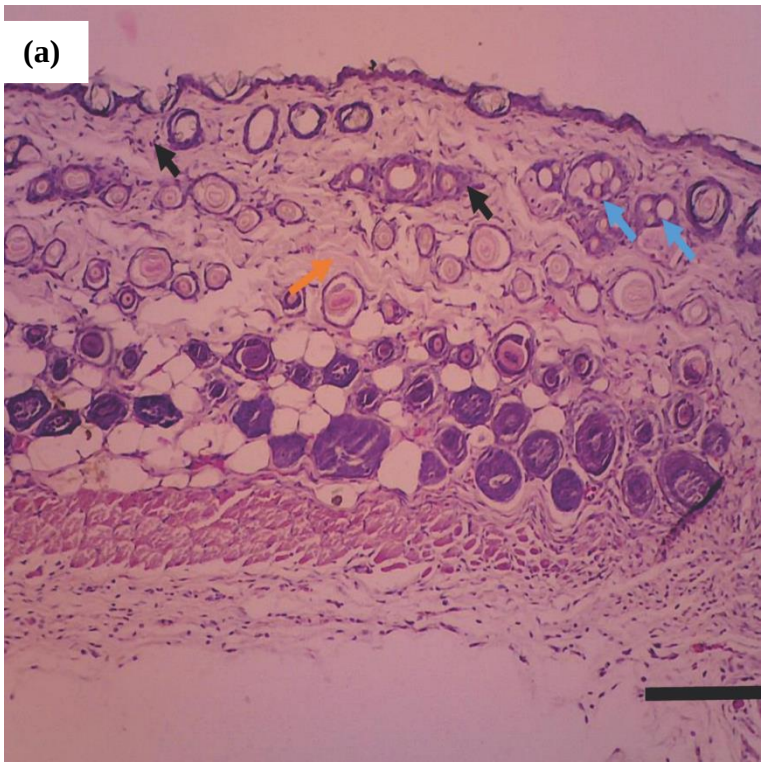
Notes:

(+) there are neocapillarisation, fibroblasts, and low-density macrophages

(++) there was neocapillarisation, fibroblasts, and macrophages with medium-density

(+++) there was neocapillarisation, fibroblasts, and macrophages with a dense density of

(++++) very dense neocapillarisation, fibroblasts, and macrophages are present



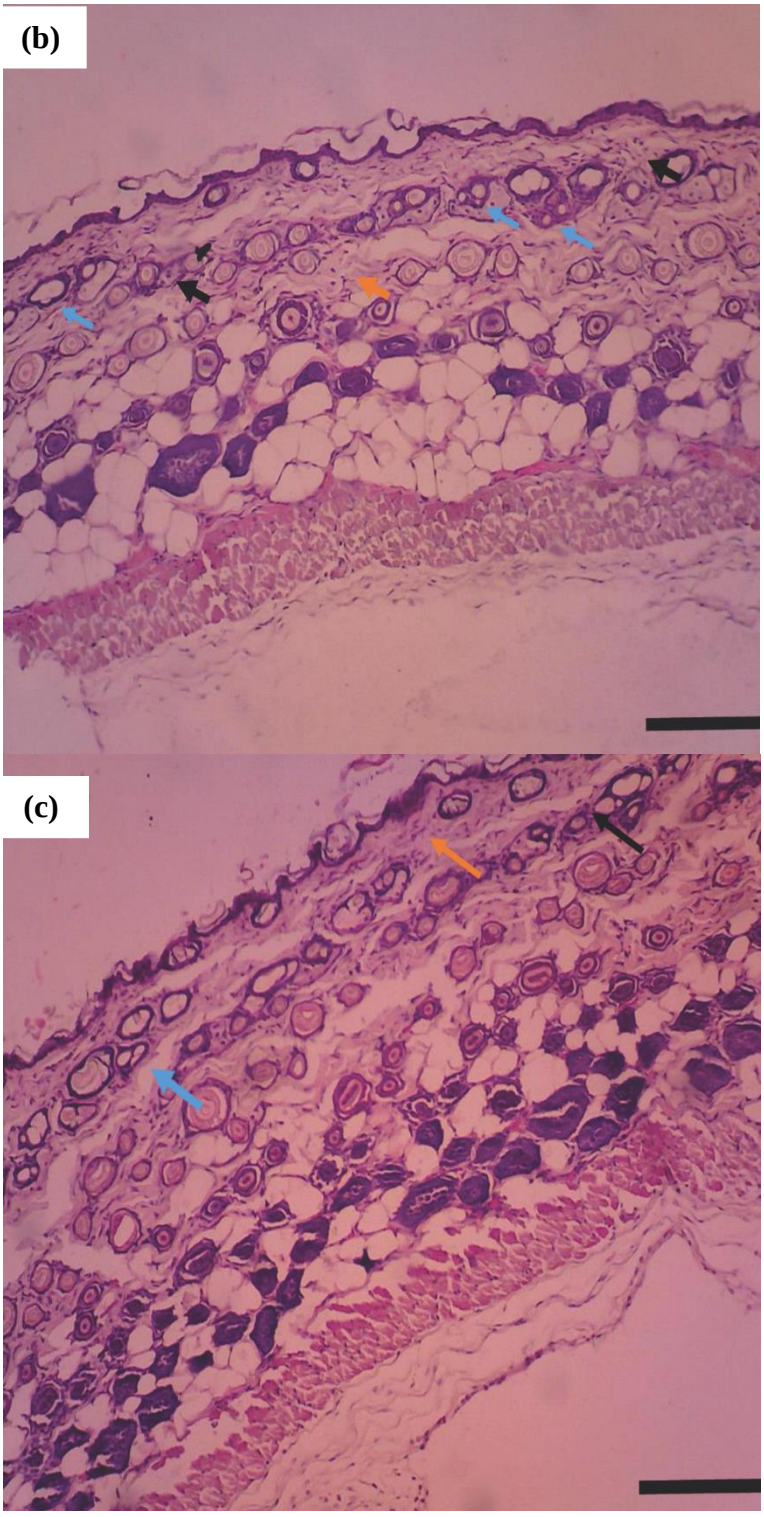



**Figure 4.** Observation of histopathological preparations of mice skin: (a) Skin tissue with NaCl, (b) Skin tissue with gelatin Broiler chicken skin, (c) Skin tissue with Bioplacenton®

Notes: = Neocapillarisation
= Fibroblasts
= Macrophages

Based on the data obtained in Table 1, shows that gelatin ointment has a longer pulling ability of 14.02 and a lower spreadability of 109.23 g.cm/second than commercial ointment (Bioplacenton®) which has a faster pulling ability of 8.60 and a higher spreadability of 178.08. According to [12] the difference in the value of spreadability greatly affects the speed of diffusion of the active substance in passing through the membrane, where the wider the membrane where the preparation spreads, the greater the diffusion coefficient which results in increased drug diffusion. The transport of molecules across membranes can be obtained through different mechanisms, usually categorized into passive or active processes. The most common mechanism for the absorption of small neutral molecules is passive transcellular diffusion, which consists of diffusion through a lipid bilayer driven by a concentration gradient. The larger the surface area of an ointment preparation, the faster the decrease in concentration that occurs (the greater the diffusion current), so that the greater the spreadability of an ointment preparation, the more it will increase its diffusion into the skin [13].

Standard doses of drug formulations are given to assess topical efficacy on the skin, where spreadability is considered to play an important role. Decreased values of ointment spreadability can be affected by the presence of a protein matrix. This indicates that the prepared base matrix easily spreads with little shear when applied to the wound surface [6]. The physical and chemical characteristics of ointment preparations are strongly influenced by the type of active ingredients used in the ointment manufacturing process. Likewise, the wound-healing process is strongly influenced by the active ingredients contained in the ointment preparation. Gelatin has been used for the synthesis of medical hydrogels due to its non-immunogenicity and capacity to enhance cell adhesion as well as its excellent biocompatibility [14]. Gelatin contains 88% protein, 10% moisture, and 1-2% salt, and on a dry weight basis, its protein content is 98-99% [15]. Gelatin has been widely used in the biomedical field because the molecular structure of gelatin contains the arginine-glycine-asparagine (RGD) series, which can promote cell adhesion and migration and make gelatin an ideal tissue repair material. Gelatin-based tissue adhesives have been reported since 1966 [16]. Based on the description of changes in mice wounds can be seen in Figures 1, 2, and 3. The healing rate of mice wounds illustrates that from day 3 to day 12 it is seen that the wounds in mice occur changes or healing. Wounds with treatment using gelatin and treatment using Bioplacenton® look faster to experience wound closure. Whereas the administration of NaCl or without treatment has not closed completely on day 12.

Data analysis obtained a significant value on the percentage of mice wound healing rate ($P< 0.05$), based on Table 2 three different types of treatment namely NaCl with a total healing value of 59.50% gives a very significant difference to the treatment of Gelatin and Bioplacenton® with a total healing percentage value on gelatin treatment which is 70.50% but does not give a significant difference to Bioplacenton® treatment where Bioplacenton® treatment has a wound healing percentage value with a total healing value of 66.25%. Based on the test results on the three types of wound treatment, it means that treatment using NaCl provides a slower wound healing process than treatment using Gelatin and Bioplacenton®. Then treatment using Gelatin is faster than treatment using Bioplacenton® but does not provide a very significant difference.

Microscopically, observations made on day 14 observed in the test group and positive control group (Bioplacenton®) the formation of neocapillarisation and connective tissue (Fibroblasts) with a density that is already dense in the blood vessels/field of view compared to the negative control group (NaCl) where the number of blood vessels from the connective tissue formed is less, namely with low density. Neocapillarisation indicates that there are many new blood vessels that will develop into new branching in the wound tissue. Blood vessels have an important role in tissue repair to provide nutrients for the regenerating tissue. An individual's physiological condition, such as an injury, can affect one's activity and performance, which requires serious treatment. The role of wound treatment by applying topical medication is a form of first aid. According to [17] the goals of first aid measures are survival, reducing pain, avoiding further illness or injury, and facilitating recovery. One of the active substances that play a role in the wound-healing process is collagen protein. Chicken skin that has a high collagen content can help accelerate the healing process or closure of open wounds due to the intake of collagen from the chicken skin. Collagen is one of the connective tissue proteins that are not water-soluble. One-third of all proteins that make up the human body are collagen [18]. Collagen stimulates biological interaction and recovery of the cell microenvironment. This collagen is a non-toxic protein and is easily absorbed by the body [19]. Chicken skin can be a new source of collagen for tissue engineering applications [19]. Gelatin itself is a product of partial hydrolysis of collagen in living things. The properties of gelatin are influenced by the properties of collagen because collagen is a derivative of fibrous proteins that have enormous benefits and roles in the formation of gelatin molecules [20]. Gelatin is a mixture of peptides derived from collagen, but supplemented by heat treatment or obtained by acid or base treatment [21]. Both collagen and gelatin are widely used as wound dressings and tissue engineering products for human use, due to their hemostatic properties, excellent biocompatibility, reduced cytotoxicity, low antigenicity, controlled biodegradability, and ability to stimulate cell attachment and growth [21], [22]. The proliferative or repair phase of wound damage is characterized by fibroplasia including proliferation and differentiation of fibroblasts into myofibroblasts, extracellular matrix deposition, wound contraction and new blood vessel formation, and peripheral nerve repair consisting of collateral reinnervation and nerve regeneration. Macrophages are the dominant inflammatory cells regulating the proliferative phase of skin wound repair [23][24][25][26].

In this study, the activity of collagen protein in gelatin towards the wound healing process showed significant results in reducing wound area and the percentage of wound healing. Gelatin affects wound healing microscopically as seen from histopathological observations and laboratory scoring results. Gelatin is a collagen dosage form in the pharmaceutical and medicinal fields that can be used topically or applied to the wound area. Gelatin is ideal because it is moist and can have an effect in accelerating wound healing. Gelatin is one of the popular forms of wound

dressing in the form of hydrogel or gelatin while the need for wound dressings for wounds is very much needed [27].

## 4. Conclusions

Gelatin has enormous potential to continue to be developed, especially in the medical field. The role of gelatin in the wound healing process shows a good healing percentage in accordance with the positive control and also has good spreading power for wounds. Histopathological observations of mice skin showed faster formation of new tissue cells compared to the negative control. Therefore, gelatin is an important molecule that needs to be developed for its sustainable use, especially in supporting the reduction of livestock waste and the advancement of medical science.

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