ORIGINAL ARTICLE

Aloe vera extract wound healing sheet and free-range chicken egg albumin (*gallus domesticus*) accelerate angiogenesis on gingival incision wound in rats

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ABSTRACT

Introduction: Incision wounds are injuries caused by dental surgery procedures. Suturing is the gold standard for post-incision management, but it can cause patient discomfort. Tissue glue can be used for wound healing, but its carcinogenicity still needs to be investigated. A Wound Healing Sheet (WHS) made of *Aloe vera* extract and free-range chicken egg albumin is expected to be an alternative material. Angiogenesis is a highly observable part of wound healing, as newly formed blood vessels support nutrition, act as a scaffold for cells to migrate to the wound area, and play an essential role in wound healing. This study aimed to analyze the effect of WHS made from *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) on the number of vascular that show the acceleration of healing in gingival incision wounds. **Methods:** Quasi experimental study with a total sample of 24 male rats (*Rattus norvegicus* Wistar strain) with gingival incision were divided into four groups, namely T1 (50% WHS treated group), T2 (100% WHS treated group, S (suturing treated group as positive control), and NC (negative control, without treatment). The treatment was performed throughout seven days. The data obtained were analyzed statistically using One-way ANOVA and Post Hoc LSD. **Results:** The highest vascular count was in the T2 Group (8+1.63); followed by T1 (7+1.60); S (7+ 1.49); and NC (4+1.41). Statistical analysis showed that there was a significant difference between groups S, T1, T2 with NC (p-value < 0.05); interestingly, there was no significant difference between S, T1, T2 (p-value > 0.05). **Conclusion:** Wound Healing Sheet made of *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) can accelerate the angiogenesis process on gingival incision wound in rats.

KEYWORDS

wound healing, aloe vera, gallus domesticus, egg white, angiogenesis

INTRODUCTION

Incision wounds are injuries caused by dental surgery procedures.[1] The incision wound needs treatment by uniting the wound edges so the tissue can function again and avoid secondary infection.[2] The suturing procedure can provide the necessary wound adhesion and is easy to perform; however, suturing can increase patient discomfort due to the pain of the needle stick, the length of time the procedure takes, and the need for a second visit for suture removal. Suturing also has a risk of secondary infection due to inflammation, as well as the risk of puncture accidents for dentists.[3]

The "tissue glue" method is an alternative non-suturing wound healing method used to overcome the shortcomings of the suturing method.[3] Tissue glue can be applied more conveniently, efficiently, and quickly compared to the suturing procedure. Tissue glue commonly found in the market today is made from 2 octyl-cyanoacrylate.[3,4] The disadvantage of tissue glue made from 2 octyl-cyanoacrylate is that it has the same risk of inflammation as suturing and its toxic and carcinogenic properties are still a topic of debate, so its use is only limited to topical application.[3] The use of tissue glue made from 2 octyl-cyanoacrylate also has several contraindications, namely for patients with gangrene, decubitus wounds, hypersensitivity to cyanoacrylate materials, vascular disease, or blood clotting disorders.[5]

Alternative materials that are more natural, safe, and minimally invasive are needed to overcome the lack of other wound healing methods. Previous research has shown that Wound Healing Sheets made of *Aloe vera* can reduce inflammation and accelerate wound healing.[6] *Aloe vera* is a natural ingredient that can accelerate post-incision wound healing and reduce the degradation rate of the sheet by water.[7] Other studies have shown the effectiveness of albumin extract gel.[8] Free-range chicken egg albumin also showed an increase in macrophage cells and accelerated the wound healing process. Albumin glue as an attachment material for bulbar conjunctiva tissue in a previous study showed a significantly stronger attachment than the suture technique on day-1 postoperatively.[9]

Aloe vera and free-range chicken egg albumin can be combined in sheet preparations to cover the oral incision area and reduce inflammation due to food residue, thereby making the post-incision wound healing process faster. This alternative can also help shorten the procedure and minimize

contact time between dentist and patient. The materials used are quite easy to find so it is expected to be developed in the future.

Our previous study has observed the effect of Wound Healing Sheets made of *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) on wound length, percentage of wound healing, and the number of fibroblasts. The result showed an increase in the percentage of wound healing and the number of fibroblasts in the group treated with Wound Healing Sheets for seven days indicating wound healing acceleration.[10] Wound Healing Sheets are made from sodium alginate and polyvinyl alcohol (PVA) with *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) is expected to be an alternative for gingival incisions wound healing. Further research on other wound healing parameters still needs to be done.

Angiogenesis is the formation of new blood vessels from old blood vessels. Angiogenesis is an essential component of wound healing since it plays a role in the supply of nutrients and oxygen to support tissue healing, promote the formation of granulation tissue, and assist in debris cleaning. Observation of the number of vascular is essential because the increase in the number of vascular in line with wound healing, therefore, can describe the acceleration of the wound healing process in the research.[11,12] This research aimed to analyze the effect of administering Wound Healing Sheets made of *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) on the wound healing process of the gingiva of Wistar rats by observing the vascular number.

METHODS

This type of research was quantitative research with in vivo laboratory experimental methods. The research design was a posttest-only control group design. Samples were taken randomly in one population, and each sample had the same opportunity to receive treatment and control. The data obtained were studied quantitatively and statistical analysis were carried out, then mean-different tests were carried out to see the effect of treatment.

The experimental animals used were 24 *Rattus norvegicus* strain Wistar rats that were healthy and without abnormalities with male sex, aged 3-4 months, with body weight 250-300 g. Exclusion criteria for experimental animals were if they were sick or had abnormalities, experienced weight loss >10%, rats experienced continuous excessive bleeding, or died. Rats were acclimatized for one week in 30 x 40 x 15 cm cages with 2 cm husks. Each cage was filled with six rats. Rats were fed with Comfeed AD II pellets according to laboratory standards and were fed ad libitum. The environmental conditions of the rats were also regulated at a temperature of 25±2 °C and humidity of 70±10%. The cage was cleaned twice a week.

Preparation of *Aloe vera* Extract, *aloe vera (Aloe vera (L) Burm*. f. or any other name *Aloe vera* var. *chinensis*) was obtained from Batu City and made into extract at the Materia Medica Laboratory in Batu Malang using the maceration method. *Aloe vera* was cleaned, cut, and dried for three days. Five kilograms of dried *Aloe vera* were mashed using a blender, put into Erlenmeyer tubes, and macerated with 1000 mL of 70% alcohol for 24 hours. The extract was filtered using a white cloth, and the liquid was heated in a water bath to obtain a precipitate of 1 L, assuming a concentration of 100%. *Aloe vera* extract with a concentration of 50% was obtained by diluting 50 mL *Aloe vera* extract in 50 mL aquadest.

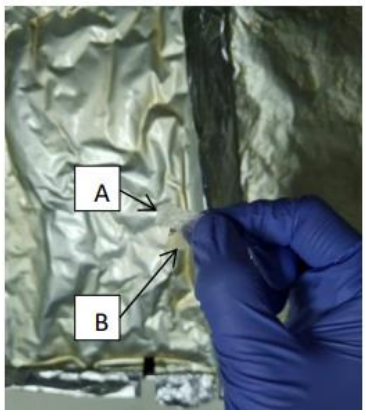


Figure 1. Results of Wound Healing Sheet Containing 100% of *Aloe vera* extract; (B) Containing 50% of *Aloe vera* extract

Wound Healing Sheets were made at the Biomedical Laboratory, Faculty of Medicine, Jenderal Sudirman University. 10 g of polyvinyl alcohol (PVA) and 1.25 g of sodium alginate were dissolved in 125 mL of aqua dm and then stirred using a magnetic stirrer for 1 hour at a speed of 1000 rpm at 25 °C. The homogeneous solution was added with *Aloe vera* extract 50% and 100% then stirred again using a magnetic stirrer at a speed of 1000 rpm at a temperature of 100 °C for 1 hour. The resulting hydrogel was poured into a tin lined with aluminum foil with a thickness of +1 mm and dried in an oven at 100 °C for 1.5 hours. The Wound Healing Sheet that had been dried was cut to a size of 5 x 7.5 mm. The organoleptic evaluation showed that Wound Healing Sheet made of *Aloe vera* extract was clear-green, and the higher the *Aloe vera* concentration, the darker the color of the

Wound Healing Sheet. The sheets were odorless and had a rigid texture. The rigidity of the sheets increased along with the *Aloe vera* extract concentrations. Wound Healing Sheet with 100% *Aloe vera* extract had a rougher and more rigorous texture but was quite elastic and did not dissolve easily. Hence, the application of the sheet is easier to do. The picture of the Wound Healing Sheet can be seen in Figure 1. The Wound Healing Sheet was then stored in a petri dish which has been sterilized and covered with aluminum foil to avoid contamination.

Rats in treatment groups were divided into four, namely T1 group, which was treated with Wound Healing Sheet with 50% extract and free-range chicken egg albumin after incision, T2 group, which was treated with Wound Healing Sheet with 100% extract and free-range chicken egg albumin after incision, S group which treated with suturing, and NC group as a negative control was not given any treatment after incision.

An incision treatment was made on the labial gingiva of the mandibular anterior teeth to facilitate access to the wound. Rats were smeared with 70% alcohol before being anesthetized and then anesthetized on the posterior thigh using zoletil intramuscularly at a dose of 0.1 mL/1kgBW until the rats were unconscious. The incision was made with a scalpel and blade no. 11 was ± 5 mm long and reached the alveolar bone.

Wound healing sheet was applied by drying the rat's gingiva, then the sheet was smeared with 0,02 mL free-range chicken egg albumin using a cotton bud and then applied to the wound using tweezers. The free-range chicken egg was obtained from Banyumas Regency. The treatment was given for seven consecutive days.

Histological preparation and observation of vascular count the rats were decapitated on the seventh day and histological preparations of the gingival incision were made with a longitudinal section. Histological staining was performed with Haematoxylin Eosin (HE). Observation of the vascular count was carried out with a light microscope with a magnification of 200x with 5 fields of view and photographed using Optilab Pro to count the vascular count. [28,29]

The statistical analysis began with the Shapiro-Wilk normality test and Levene's homogeneity test (p-value > 0.05). The One-Way ANOVA test was carried out with a significance of 95% (p-value = 0.05), followed by the Post Hoc Analysis using the Least Significant Different (LSD) test. P-value < 0.05 was considered statistically significant.

RESULTS

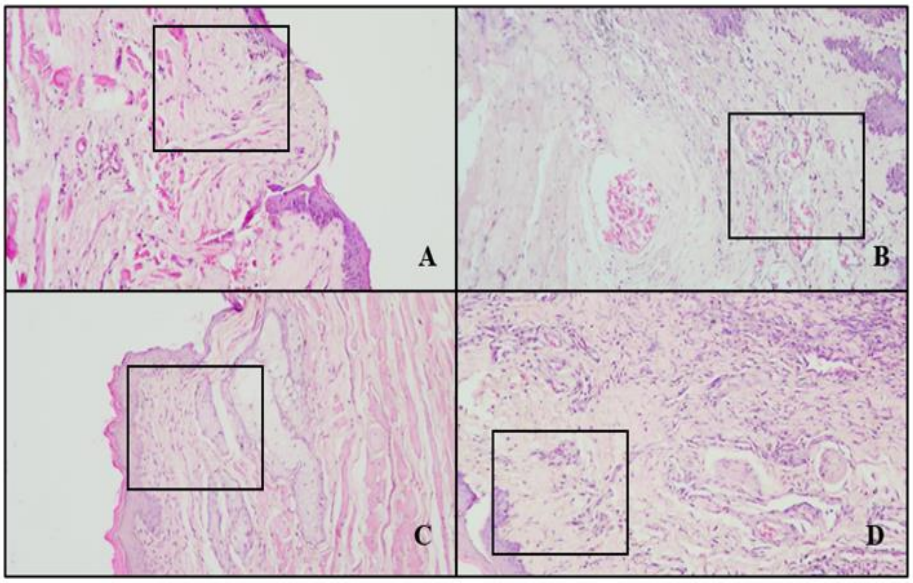


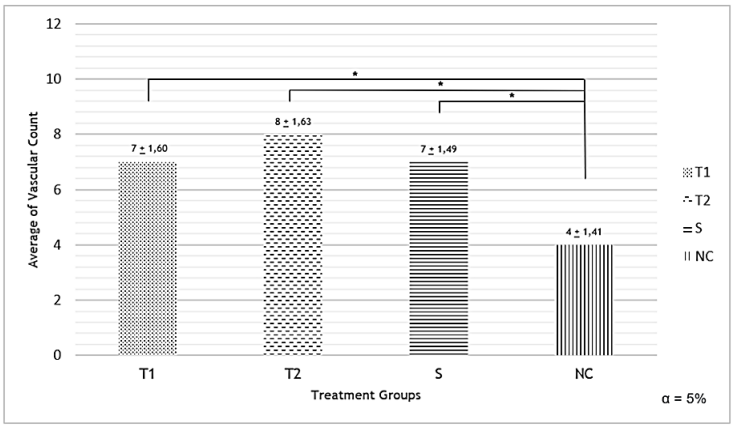
Figure 2. Histological features of vascular cells in the treatment group: T1; (B). T2; (C). S; (D) NC (200x magnification)[28,29]

Vascular cells observed at 200x magnification can be seen in Figure 2. Histological features of vascular cells are seen to have a simple squamous epithelium layer with a slightly circular shape and a lumen in the middle. Connective tissue cells called pericytes can also be seen at the margins of the lumen beneath the endothelial cells.

Table 1. Vascular count (mean ± SD), observation on day 7

Treatment Group	n	Mean ± SD
T1	6	7 ± 1,60
T2	6	8 ± 1,63
S	6	7 ± 1,49
NC	6	4 ± 1,41

Table 2. Post Hoc analysis of vascular count (mean ± SD)



Description:
T1: Wound Healing Sheet with 50% *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) was given after the gingival incision. T2: Wound Healing Sheet made of 100% *Aloe vera* extract and free-range chicken egg albumin *(Gallus domesticus*) was given after the gingival incision. S: suturing treatment after gingival incision. NC: The negative control was not given any treatment after the gingival incision was made *: Post Hoc analysis (P-value < 0,05).

In the vascular count data, it can be observed that the highest vascular count was in the T2 group, and the lowest was in the NC group. The vascular count in the T1 group using the 50% extract was 7 + 1.60, and the T2 group using the 100% extract was 8 + 1.63. As the level of *Aloe vera* used increased, the mean vascular number also increased.

The One-Way ANOVA test results showed a significant difference in vascular count between groups (p-value < 0.05). Post hoc LSD analysis showed a significant difference in the mean of vascular count between groups S, T1, T2 with NC (p-value < 0.05). However, there was no significant difference between S, T1, T2 (p-value > 0.05). It indicated that the Wound Healing Sheet significantly increased angiogenesis compared to the control group. The effect was comparable to the suturing method, which was the gold standard method for wound closure.

DISCUSSION

The results showed a significant increase in the number of vascular in the provision of Wound Healing Sheets compared to the negative control group (p-value < 0.05). It indicates an increase in angiogenesis on the 7th day after treatment with Wound Healing Sheet made of *Aloe vera* extract and free-range chicken egg albumin. This is in accordance with the research of Mogosanu et all., who observed the healing of third-degree skin burns treated with topical preparations containing herbal extract. This study showed the results of wounds treated with topical preparations containing herbal extracts can increase the number of vascular due to the flavonoids and tannins present in herbal extracts.[6,13]

In our study, we also used films made from herbal extracts, namely *aloe vera* and free-range chicken egg albumin, where the substances contained in the herbal extracts can accelerate the angiogenesis process. In a study conducted by Koga et al., regarding wounds treated using alginate films containing Aloe vera gel and zinc chloride, also showed the results that aloe-alginate films can enhance the healing process of incisional skin wounds due to the nature of the film covering the wound as well as tannins, flavonoids, acemannan, and other ingredients contained in aloe-alginate films.[6,13]

Based on the analysis results, incision wounds on the gingiva of Wistar rats that were given Wound Healing Sheet treatment had a vascular count that was not significantly different from the incision wounds that were treated with suturing (p-value> 0.05). It means that Wound Healing Sheet made of *Aloe vera* extract and free-range chicken egg albumin can be used as an alternative to suturing. This study also showed no significant difference between the mean vascular count of 50% WHS *Aloe vera* extract and 100% WHS *Aloe vera* extract (p-value> 0.05). The previous research on the effect of Wound Healing Sheets of *Aloe vera* extract and free-range chicken egg albumin on wound length, healing percentage, and the number of fibroblasts also yielded similar results. The group that was given a Wound Healing Sheet with *Aloe vera* extract and free-range chicken egg albumin had a smaller wound length than the positive and negative control groups. The percentage of wound healing in the group given Wound Healing Sheets showed results that were not significantly different from the gold standard for wound closure, namely suturing. The number of fibroblasts in the group that was given Wound Healing Sheet also had significantly different results from the positive and negative control groups. There was no significant mean number of fibroblasts between the groups using 50% and 100% extracts, meaning that the use of 50 and 100% *Aloe vera* extract has the same effectiveness in treating post-incision wounds and accelerating wound healing processes.[10]

Angiogenesis is the process of the formation of new blood vessels from pre-existing blood vessels that migrate and proliferate to elongate blood vessels. Adjacent blood vessels will join and form a bloodstream. Non-perfused blood vessels will regress.[14] Angiogenesis plays an essential role in tissue regeneration or tissue repair by providing sufficient nutrients, oxygen, and various growth factors.[15] Angiogenesis is one of the stages in the wound healing phases. The wound healing phase is divided

into three phases, namely the inflammatory phase, the proliferative phase, and the maturation phase. The initial inflammatory phase begins with hemostasis which stop bleeding, followed by phagocytosis of bacteria and debris. [16]

The inflammatory phase occurred from day 1 to day 3 after wound healing occurred. The next phase of wound healing was the proliferative phase which functioned to cover and fill the defect area, as well as restore skin and mucosal function. In this phase, the process of angiogenesis occurred. The proliferative phase runs 2-4 weeks after injury. The final phase of wound healing is the maturation phase, which strengthens the defective tissue's tensile strength. The maturation phase occurred one week after injury and can last for more than a year.[14,15,16]

Large and deep injuries required tissue union to facilitate the epithelialization of wounds and connective tissue. It also served to reduce infection rates.[16] The NC group described the condition of untreated incisions that took longer to heal because more time was required to close and fill the defect area. This healing also had a greater risk of infection because there was no barrier to the lining epithelium. Infections due to bacteria or debris and additional trauma due to rubbing with the tongue or food scraps could also make the inflammatory phase last longer and hamper the process of angiogenesis and transfer of oxygen and nutrients. [16,18]

Treatment groups of T1 and T2 were applied with Wound Healing Sheets containing *Aloe vera* extract and free-range chicken egg albumin whose active ingredients could accelerate angiogenesis and wound healing. *Aloe vera* has been used for therapeutic purposes for ages. Its value for moisturizing skin, accelerating wound healing, and treating heat-related skin injuries, frostbite, and ischemic wounds to the skin is supported by clinical and experimental evidence. *Aloe vera* has been studied in numerous studies and different experimental models for its anti-inflammatory, vasodilatory, antimicrobial, and proliferative properties. As in the study by Mohammed et all., that looked at how aloe vera affected the recovery of rabbits' maxillary mucosal wounds after surgery. The findings of Mohammed et all., are consistent with this study, which demonstrates that the use of *Aloe Vera* gel positively impacts the healing process by promoting the development of granulation tissue, which in turn promotes angiogenesis and increased re-epithelialization. [19] *Aloe vera* had active substances including flavonoids. Flavonoids are useful as antibacterial, antioxidant, anti-inflammatory, and analgesic. Kaempferol, which is one of the flavonol compounds in *Aloe vera* bound vascular endothelial growth factor (VEGF) which is the main angiogenetic factor to potentiate the angiogenesis process.[15]

Aloe vera contains acemannan, which serves as *Aloe vera*'s primary functional ingredient. It is a complex carbohydrate with an extensive chain of acetylated mannose. Recent research has shown the significance of the acetyl group and its relationship to VEGF (vascular endothelial growth factor) expression in cells. According to studies, acemannan promotes the growth of granulation tissue by increasing cell division and VEGF activity, two factors crucial for the development of new blood vessels and the proliferation and migration of endothelial cells. Additionally, by binding to the mannose receptor on macrophages, acemannan may promote the release of fibrogenic cytokines, which in turn promotes the recruitment of fibroblast cells and the formation of granulation tissue. Acemannan also increased the proliferation of fibroblasts and the release of keratinocyte growth factor-1 (KGF-1), and collagen synthesis type I.[6] *Aloe vera* also contained anthraquinones, saponins, tannins, and polyphenols, which acted as antibacterial and anti-inflammatory so they could accelerate wound healing. Tannins contained in *Aloe vera* could also function as antioxidants and antiseptics.[10,19,21]

Free-range chicken egg (*Gallus domesticus*) contains albumin which is important in wound healing due to its contents of natural proteins and collagen, which form the extracellular matrix and induce growth factors, as well as tissue regeneration. Ovalbumin is the most abundant protein in free-range chicken eggs. Ovalbumin plays a role in cell migration and supports cell attachment and adhesion so the egg whites can be used as albumin glue. Previous studies showed that tissue adhesion in the albumin glue technique is denser than in the suture technique because in the suture technique there is the possibility of retraction so the tissue does not stick flat on the bottom.[9,10] Lysozyme in the albumin of a free-range chicken egg has a solid inhibitory power against bacteria to protect the tissue from bacterial infection.[8] Free-range chicken egg albumin also contains lysophosphatidic acid which is found in egg whites and can induce the formation of blood vessels by increasing VEGF expression. VEGF is involved in many stages of the angiogenic response, including stimulating proliferation, migration, survival, permeabilization of endothelial cells, and stimulating the degradation of the extracellular matrix surrounding the endothelium to accommodate blood vessel branching.[22]

The results showed a significant difference in the mean vascular count between groups S and NC but not significantly different between groups S and T1 and T2. This study shows different results from the research conducted by Milwati and Retnaningtyas where 73% of respondents who were sutured experienced signs of inflammation that could cause a slowdown in healing.[23] The use of suturing methods and materials depended on the wound condition. The technique most commonly used for mucosal surface suturing was simple interrupted sutures. This technique had high tensile strength with little potential for oedema and circulatory disturbance. However, it took longer to do. In addition, this technique tended to leave linear scars resembling railroad tracks and caused wound inversion if not appropriately placed.[23] The choice of suturing material could also affect wound healing.[24] The absorbable sutures were a type of sutures that the body's enzymes can digest. These sutures provide several advantages, such as avoiding the patient's discomfort when it is necessary to take sutures. However, because the absorption of these sutures involves enzyme digestion and phagocytosis, absorbable sutures often cause inflammation. Another type of suture material was non-absorbable sutures.[24] This type of suture could not be digested by enzymes or hydrolyzed by the body. These sutures had great strength and stably hold the tissue. However, non-absorbable sutures

could become foreign bodies that remain in the body and might lead to fistulas. In addition, this type of sutures should be opened in the first week after being installed because it could cause plaque buildup and accumulation making it easier for bacteria to enter the wound and cause infection and inflammation. [24]

Incision wounds on the gingiva of Wistar rats who were administered Wound Healing Sheets made of *Aloe vera* extract and free-range chicken egg albumin, based on the results of the analysis, had the number of vascular that was not significantly different from the positive control group, namely the incision wound that was treated with suturing (p-value > 0.05). It means that Wound Healing Sheets can be used as an alternative to suturing because they had almost the same angiogenesis effect as the group that was treated with suturing. Suturing can caused mechanical wound closure so that the infection risk could be reduced, and the closure of the wound edges could be accelerated.[25] Suturing functions as a temporary scaffold that provide mechanical support for the wound during the acute stage.[26] Wound Healing Sheet made of extract of 50% or 100% was also used to cover the wound to reduce the risk of infection and tissue trauma and accelerate regeneration and angiogenesis. The results of previous research where the wound was administered a film based on alginate and PVA and containing *Aloe vera* showed a film with adequate mechanical resistance and flexibility as a wound dressing.[6,27]

Aloe vera made in sheet preparations had elastic and flexible properties to make Wound Healing Sheets painless when installed or removed. Wound Healing Sheets with 50% extract had a softer and smoother texture. However, they tended to bend easily and dissolved more easily, making it difficult to apply to the mucosa of experimental animals. The ethanol solvent used to dilute the 50% extract made all PVA dissolve, so the 50% concentration became smoother and easily dissolved. Wound Healing Sheet with 100% extract had a rougher and more rigid preparation but was quite elastic and did not dissolve easily when applied to experimental animals. Hence, the application and removal of the mucosa of experimental animals were easier.

For further research, it is possible to further explore the effective dose by using a smaller concentration. There was also only one parameter of wound healing observed in this study. There are many other wound healing parameters, so it is possible to conduct this research on other wound healing parameters. A toxicity test can also be carried out to detect the toxic effects of this Wound Healing Sheet.

CONCLUSION

Wound Healing Sheet made of *Aloe vera* extract and free-range chicken egg albumin (*Gallus domesticus*) can accelerate the angiogenesis process on gingival incision wound in rats.

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Informed Consent Statement: Not applicable

Data Availability Statement: The availability of research data will be provided with the permission of all researchers via correspondence email with due regard to research ethics

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