EFFECT OF BAKIKO (SPINACH-CHITOSAN-COLLAGEN) MEMBRANE IN BURNS HEALING

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Abstract: Background: Burns are a global public health problem, accounting for an estimated 265.000 deaths annually. Burns injuryneed a complex wound treatment because they are opened wound that damage epidermis and tissues below and it stimulates the process of inflammation, infection, and on the severe condition, evaporation of body fluids followed by heat and energy lost. Local treatment of burn injury must have an effect as antimicrobial, anti inflammation, antioxidant, and give moist condition for the wound that can accelerate healing process.Spinach, Chitosan, and Collagen are use full in wound healing process as antiinflammation, antioxidant, and antimicrobial.
Materials and Methods: This research uses quasi experimental in vivo with post test only randomized control group design. This study were divided into 4 groups, each group contain nine rats. Control is a group that is not given any treatment. Positive control group is a group that given burn wound plus bioplacenton. Negative control group is a group that given burn wound. Treatment group is a group that given burn wound plus BaKiKomembrane. We observed the macroscopic process of burn healing. We also measure burn's areas with ImageJ software.
Result: BaKiKo membrane treatment in the treatment group and Bioplacenton in the K+ (positive control) group can improve the burn wound healing progression compared to the K- (negative control) group. This is supported by statistical analysis which shows that the data obtained show significant data because p <0,05.
Conclusion: Combination of spinach, collagen, and chitosan can accelerate the progressive healing of burns because it can stimulate collagen synthesis.

Keywords: ***burn healing,chitosan, collagen, spinach***

I. BACKGROUND

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Skin injuries due to ultraviolet radiation, radioactivity, electricity or chemicals, as well as respiratory damage resulting from smoke inhalation, are also considered to be burns. Burns are a global public health problem, accounting for an estimated 265.000 deaths annually. The majority of these occur in low- and middle-income countries and almost half occur in the WHO South-East Asia Region. Non-fatal burns are a leading cause of morbidity, including prolonged hospitalization, disfigurement and disability, often with resulting stigma and rejection (World Health Organization, 2016)[1].

The etiologies of burns vary across different age groups. A study that examined work injuries across age groups found adolescents had a rate of permanent impairment as a result of amputations, burns and cuts higher than that of adults[2].The most common mechanism of burn injury was flame (32.7%) followed by electrical (27%), scald (19.7%), chemical (10.3%), tar (5.2%), and contact (5.1%)[3].According to the National Burn Repository, flame burns and scalding injuries account for 80 percent of etiology of burns, which mainly occur in domestic environments.

Burns are among the most complex form of injuries. Burn injuries can cause both physical and psychological trauma. Physically, burns can cause pain, sensory problems, and weakness. Patients with burn injuries become immunocompromised because of their wounds and systemic changes, which can elevate the risk for developing various complications such as sepsis, respiration failures, shock and death. Beyond physical complications, burns can also result in severe psychological and emotional distress because of long-term hospitalization, scarring, and deformity.

Burn injury divided into 3 grade, which is grade I (epidermal), grade II (partial thickness), and grade III (full thickness). All of those grade (except grade I), need a complex wound treatment because they are opened wound that damage epidermis and tissues below and it stimulates the process of inflammation, infection, and,on the severe condition, evaporation of body fluids followed by heat and energy lost. This is why, burn injury need special and fast treatment. Local treatment of burn injury use ingredients such as antimicrobial, anti inflammation, antioxidant, and give moist condition for the wound that can accelerate healing process.

Spinach is rich in vitamins and minerals. Effective compounds in spinach include zinc, glutamine (Gln), arginine (Arg), and vitamins A, C, E[4]. Glutamine and Zinc involved in the proliferation of cells. Lack both of Glutamine and Zinc could affect the critical roles that these processes play in healing wounds resulting in a delay of wound healing [5,6]. Several studies show that arginine supplementation accelerates wound healing [7]. In wounds, catabolizes of arginine is done in two distinct routes: nitric oxide synthases and arginase. L-Arginine is transformed into ornithine using arginase, and ornithine acts as a substrate for proline and polyamine production. It has

been hypothesized that this route maybe one of the important paths towards healing, because proline, as a substrate, helps polyamine synthesis, whereas collagen assists the process of cell proliferation [8]. For a normal wound healing, sufficient protein intake is essential because collagen synthesis is inhibited when insufficient protein is taken. Amino acids such as Gln and Arg are required for optimal collagen synthesis. It has been found that abundance of vitamin A in spinach is useful for the wound healing process as it stimulates epithelization and collagen deposition by fibroblasts. Spinach is also high in vitamin C, which increases the formation of granulation tissues (such as fibro vascular tissues that contain fibroblasts, collagen, and blood vessels) that characterizes the start of the healing reactions. Vitamin E maintains the membrane mainly through protection of cell membranes against the destruction caused by oxidation which may influence the diverse functions of the host immune system and reduce damage to the wound.

Collagen is a significant constituent of the extracellular matrix (ECM)[9]. We combined collagen due to a number of useful properties, such as homeostasis effect, low antigenicity, good biocompatibility, high mechanical strength for use in soft tissue engineering applications[9-13]. Collagen has been widely applied in tissue engineering applications and in some extent in delivery systems in this field[12]. Cells in a tissue are surrounded with other cells and ECM. ECM provides chemical and mechanical signals whose effects are interdependent. Chemical signals may originate in the chemical structure of ECM components or may be provided by cytokines and growth factors stored in the ECM and released under certain circumstances. ECM is degradable and cells can migrate through it [14]. Collagen is an excellent attachment substrate for cells, which can recognize and bind to the protein through integrin receptors. Cells not only attach to collagen but can also degrade it by secreting specific enzymes and can synthesize new collagen via intracellular production and export to the extracellular space. In this way, cells remove, remodel, and replace collagen, a process that is important in the homeostasis of many tissues and organs[9]. Fibroblasts and myofibroblasts are cells involved in the healing of various tissues. Fibroblasts in the tissue surrounding the wound are activated and migrate into the provisional matrix containing fibrin and plasma fibronectin. Fibrin is a major component of the provisional matrix formed during wound healing and enables migration of inflammatory cells and fibroblasts. Collagen production becomes the main fibroblast function and the provisional matrix is gradually replaced with a collagenous ECM [14]. Morphogenesis, tissue remodeling, and tissue repair all require the targeted remodeling of interstitial and basement membrane collagen to allow for organ growth, cell migration, and translation of contextual cues that are embedded within the extracellular matrix.

Chitin/chitosan is produced from the exoskeleton of sea food, shellfish, crabs, shrimps, insects, edible mushrooms, and sea weed algae[15]. Chitosan as natural cationic polysaccharide is composed of glucosamine (GlcN) and n-acetylglucosamine (GlcNAc) residues and serves an important role in wound healing and regenerative medicine. It is a biodegradable polysaccharide, which is degraded slowly by lysozymes, chitinase, and chitosanase into harmless oligomers that significantly activate the wound healing process.The other advantages are that chitosan resembles with various glycosaminoglycans, which are distributed throughout the human body and are essential for cell morphology and differentiation, provides a suitable microenvironment for wound healing and cellular proliferation by behaving like an extracellular matrix and aids in tissue growth, initiates proliferation of fibroblast cells, and stimulates collagen synthesis. It also possesses antimicrobial properties against a variety of bacteria, fungi, and algae, preventing wound infection[16]. In biochemical activities of chitin/chitosan-based materials include: anti-infectious activity; stimulation of angiogenesis/wound repair; and stabilization/activation of growth factors[17]. Growth factor increases cell proliferation, diferentiated cell and angiogenesis[18,19].

Based on previous experiment mentioned above, Spinach, Chitosan, and Collagen are use full in wound healing process as anti-inflammation, antioxidant, and antimicrobial. But there were no experiment that combined the three of them in membrane form.

II. MATERIALS AND METHODS

Research design

This research uses quasi experimental in vivo with post test only randomized control group design which will be conducted in Pharmacology Laboratory Faculty of Dentistry, University of Jember, Histology Laboratory Faculty of Dentistry, University of Jember, and Molecular Biology Laboratory Faculty of Medicine, University of Jember.

The sample of this research was *Rattusnorvegicus* male wistar rats from Parasitology Laboratory of Brawijaya University placed in plastic cage with husk base and fed with pellet and given drink *ad libitum*. Inclusion criteria include *Rattusnorvegicus* male wistar rats, 2-3 months old with weight 150-200 grams, healthy, active and normal moves. The exclusion criterion is the *Rattusnorvegicus* male wistar rats which during the study did not want to eat and die. The license of ethical clearance on the research procedure is submitted to the medical research ethics commission of the Faculty of Medicine, University of Jember.

This study were divided into 4 groups, each group contain nine rats. Control is a group that is not given any treatment. Positive control group is a group that given burn wound plus bioplacenton. Negative control group is a group that given burn wound. Treatment group is a group that given burn wound plus BaKiKo membrane. In this research, we observed the macroscopic process of burn healing. We also measure burn's areas with ImageJ software.

Spinach Extraction

Spinach (*Amaranthus sp.*) that identified by Agronomy Laboratory in Jember University is extracted using aquadest at a ratio of 1: 1. Then filtered using a tofu filter and extracted. The last stage is freeze drying to get the extract of dried spinach.

Spinach-Chitosan-Collagen Membranes

Collagen solution with a concentration of 0.41 mg/ml was mixed with 1% chitosan in 0.5M acetic acid. Then, add the spinach extract. The membrane is molded by pouring the spinach-chitosan-collagen composite into a glass plate with a thickness around ± 1 mm. Then we let stand for 24 hours at room temperature. The dried bioplastics are inserted in a coagulant tube containing 4% NaOH to help removing the membrane. BaKiKo membrane will be attached to the burn and replaced every 3 days.

Animal Preparation

Animals is under anesthesia with ketamine 0.1cc intramuscularly. The back skin is shaved and thoroughly depilated. Burns were made as contact burns with 2cm metal plate diameter rounds (105˚C, 5 seconds) on the right and left sides of the rat's back. One hour after burning, the control positive and treatment group was given therapy.

Observation of Burn Injury

Wound macroscopic observation was performed on the 3rd, 7th, 10th, 14th, 17th and 21st days. In this observation, measured the progress of wound healing. Measurements are made by drawing and photographing the wound surface. Furthermore the image is processed by using ImageJ software.Later, the healing ratio was calculated by the following equations:[4]

$$\text{healing ratio}(\%) = 100 \times \frac{wound\ area}{initial\ wound\ area}$$

Data Analysis

We analyzed wound surface and burn's area. They were normality test and variance test. If the distribution of normal data and variant data is the same (p> 0,05), data analysis used is One Way Anova. However, if not the same (p <0.05) the Kruskal Wallis test is used. Furthermore, Post Tukey's test was used as an advanced One Way Anova and Mann Whitney as a further test of Kruskal Wallis to determine meaningful differences in each group. Differences per group were assessed to be significant if the p value <0.05.

III. RESULT

Burn Healing Progress

Measurement of widespread burns is done on days 3th, 7th, 10th, 14th, 17th, and 21th. The width of the wound is drawn and photographed for subsequent calculations either manually or using ImageJ software. Groups that can provide an overview of burn wound healing progression are K+21, K-21, and P21.

In this study, data on negative control was used in establishing normal healing progress in rats. Negative control is a group that given burns only, so it can be used as a reference of normal wound healing.Data on possitive control was used in establishing healing progress in rats that given by common treatment of burn injuries. Possitive control is a group that given burn wound plus bioplacenton, so it can be used as a reference of wound healing with common treatment.

Group	Wound Area (%)					
	H3	H7	H10	H14	H17	H21
K-	100.00	75.52	58.17	40.29	28.99	8.49
K+	100.00	66.76	46.54	20.55	10.68	0.00
P	100.00	87.42	63.01	27.28	0.61	0.00
P value *<0.05	0.81	0.27	0.14	0.12	0.03*	0.02*

Table 1. Burn Healing Progress

Based on the data obtained, BaKiKo membrane treatment in the treatment group and Bioplacenton in the K+group can improve the burn wound healing progression compared to the K-group. This is supported by statistical analysis which shows that the data obtained show significant data because p <0,05(p=0.03 and p=0.02). It can be concluded that BaKiKo membrane administration can improve the burn wound healing progressivity.

BaKiKo membrane consist of aqueous extract spinach, chitosan powder, and collagen powder. Each material have a different function in the wound healing of burn injuries. Spinach have a lot of advantages, Gln and zinc involved in the proliferation of cell, Arg can accelerate wound healing, Gln and Arg support collagen synthesis, vitamin A also stimulates epithelization, vitamin C increases the formation of granulation tissue, and vitamin E can against the destruction caused by oxidation. Chitosan significantly activate the wound healing process, initiates proliferation of fibroblast cells, stimulates collagen synthesis and preventing wound infection (antimicrobial). Added collagen powder can make value of collagen increase and it can stimulate synthesize new collagen.

Bioplacenton is a topical gel-formed drug packed in tubes. Bioplacenton consist of 0.5% neomycin sulfate content and 10% placenta extract. Placenta extracts present in this material can stimulate cell regeneration, whereas neomycin sulfate may act as a bactericide. In K+ group wound healing was finished

at day 21while rats in K- group wound healing wasn't finished at that day.Almost of rats in treatment group (P group) was finished at day 17, faster than K+. So, we can conclude that wound healing of treatment group is faster than K-group andK+group.

CONCLUSION

BaKiKomembrane has potential as a burn therapy. Combination of spinach, collagen, and chitosan can accelerate the progressive healing of burns because it can stimulate collagen synthesis. BaKiKo is designed in the form of plaster so it is easily applied to the wound. Further research is needed to know the effective dose and toxicity of BaKiKo.

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