Indonesian Journal of
Tropical and Infectious Disease
Vol. 5. No. 1 January–April 2014

Research Report

THE UTILIZATION OF *ACHATINA FULICA* MUCUS IN ALGINATE MEMBRANE AS WOUND HEALING ACCELERATOR AND ANTI-INFECTION MATERIAL

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ABSTRACT

Wound should be covered with bandage that is called wound dressing. Most people use synthetic materials such as gauze dressing. Gauze has high absorption of NaCl, which is often used to cleanse the wound. However, discomfort and pain arise since the gauze becomes sticky on the wound. Therefore, we need other alternatives instead of gauze to cover wound. One such alternative is the alginate membrane. This study used alginate membrane with mixture of mucous of the snail Achatina fulica, which contain proteins such as proline, serine asparagine, glycosaminoglycan, hydroxylysine, trionin and so forth, to activate the growth factor. Alginate powder and carboxymethl cellulose (CMC) was dissolved in distilled water mixed with mucus of the snail Achatina fulica in four variations (4:0; 4:1, 4:2, 4:3) through a magnetic stirrer, and casted on a baking sheet covered with sterile gauze. High Performance Liquid Chromatography (HPLC) test showed that the glycosaminoglycan content was found on the mucous of Achatina fulica. This was indicated by the appearance of peak at 325–350 second. The most optimum alginate and mucus composition was in ratio of 4:2. This ratio resulted in a wound dressing that was still able to absorb the exudate and optimally accelerated wound healing.

Key words: *alginate, Achatina fulica, wound healing accelerator*

ABSTRAK

Luka seharusnya ditutup dengan perban yang dinamakan dengan penutup luka. Banyak orang menggunakan material sintetik seperti penutup kasa. Kasa memiliki daya serap NaCl, yang biasa digunakan untuk membersihkan luka. Bagaimanapun, ketidaknyamanan dan rasa sakit timbul ketika kasa menjadi lengket pada luka. Oleh karena itu, kita membutuhkan alternative kasa untuk menutup luka. Salah satunya ialah membran alginat. Pada penelitian ini menggunakan membran alginat yang dicampur dengan lender bekicot Achatina fulica,yang mengandung protein seperti proline, serine asparagines, glycosaminoglycan, hydroxylysine, trionin, dan sebagainya untuk mengaktifkan growth factor. Bubuk alginat dan carboxymethl cellulose (CMC) dilararutkan pada air suling dan dicampur dengan lender bekicot Achatina fulica dengan empat variasi komposisi (4:0; 4:1, 4:2, 4:3) menggunakan magnetic stirrer dan diletakkan pada tempat oven dengan dilapisi kasa steril. Uji High Performance Liquid Chromatography (HPLC) menunjukkan bahwa kandungan glycosaminoglycan ditemukan pada lendir bekicot Achatina fulica. Hal diindikasikan dengan munculnya puncak pada detik 325-350. Alginat yang paling optimum dan dan lendir pada komposisi 4:2. Komposisi ini menghasilkan penutup luka yang tetap bisa menyerap nanah dan optimal dalam mempercepat penyembuhan luka.

Kata kunci*: alginate, Achatina fulica, percepatan penyembuhan luka, , glycosaminoglycan, carboxymethl cellulose*

INTRODUCTION

Traffic accident is one of the causes of high mortality in Indonesia. Based on data from the Jakarta Police Department, the number of accidents during 2010 reached 8059 cases, from which the number of people killed was as many as 1,032, seriously injured 3,429 people, and minor injuries 5,679 people. Data toward the end of 2011 stated, 8,468 victims of accidents in and around Jakarta, as many as 11.04% died. A total of 2,241 people were seriously injured and those with slight injury were as many as 5,292 people (62.49%).[1] Each accident victim requires treatment to his injuries. By default of wound management, the wound should be covered with a membrane or a bandage called the wound dressing. Usually people use a synthetic form of gauze dressing as wound dressings. The gauze is keeping the wound from the surrounding trauma. However, the gauze quickly absorbs NaCl which is previously used to wash wounds. This raises the patient's discomfort and worry if infection may occur due to sticky gauze on the wound.[2]

There are some research that conducted an experimental study comparing the use of conserved amnion with synthetic wound dressing material coated with gauze to cover circumcision wound in 16 children. From the results, it can be concluded that the use of conserved amnion as a circumcision wound closure is more effective in reducing pain when removing dressings circumcision and can reduce the risk of infection in treating circumcision wound, compared to the use of gauze coated synthetic materials on the outside. Besides gauze, wound dressings as amniotic membrane and membrane alginate have been widely used today.

Amnion has great benefits as a wound cover because it contains growth factors that help natural process of cell proliferation. However, not all pregnant women and their families allow to donate the placental membranes to take the amnion, so that the source of the amnion becomes very limited.[3] Therefore, we need another alternative as a substitute for the amnion to function as wound closure. One alternative is the alginate membrane. Currently, the widely used wound closure is pad or wick-shaped alginate. Imported foam products are typically that of hydrogel material. Based on studies, it is known that the foam or sponge can be made as alginate material that have a high absorption of wound containing liquid such as exudate. To accelerate wound healing, the membranes are usually given with medication or substance that could cause more active growth factor in human skin. The main element that can activate the growth factors are proteins such as proline, serine asparagine, hydroxylysine, trionin and so forth.[4] The material contained in one species *Achatina fulica* snail mucus.

During this time, snails are only be used as a food ingredient in the form of chips or sate. Moreover, villagers apparently use the mucus from these molluscs as toothache medicine. It is less hygienic. Therefore, we need the development of research on snail mucus to be used as a wound healer.[5] The combination of alginate, carboxymethyl cellulose (CMC) as a thickener, and snail mucus, is a solution to the needs of eco-friendly wound closure membrane and accelerate wound healing that is expected to reduce the incidence of wound infection and treatment costs.

MATERIALS

Materials used in this study were *Achatina fulica* snail mucus, sodium alginate with brand Sigma Aldrich 71238, and carboxymethyl cellulose (CMC) of Brataco technical types.

METHODS

Early Preparation

The first stage was the process of snail mucus snail mucus removal by preparing some snails from which the mucus would to be removed. Then, part of the shell was washed with water until clean. After that, the tip of its taper-shaped shell was cut about 0.5 cm.

Samples Preparation

Alginate membranes – snail mucus – carboxymethyl cellulose (CMC) was made from mixing powdered sodium alginate and CMC that had been diluted with a solvent, such as distilled water, and snail mucus. Each solution was mixed and stirred with a magnetic stirrer in order to be homogeneous. This solution was then casted on a round baking pan that has been coated with sterile gauze, and then stored in a deep freezer with a temperature range of –80°C to –100°C for ± 24 hours. After 24 hours in the freezer, the samples were removed and immediately lyophilized for 72 hours at a temperature of about –105°C and pressures in miliTorr. There were four variations of composition in this study using a total weight of 1% (Table 1).

Table 1. Composition Variations

Sampel	Alginate: Achatina fulica Mucus	Alginate (gr)	Distilled water solvent (gr)	Achatina fulica mucus (gr)
A	4 : 0	1.4	140	0
B	4 : 1	1.1	110	2.8
C	4 : 2	0.92	92	4.6
D	4 : 3	0.80	80	6.0

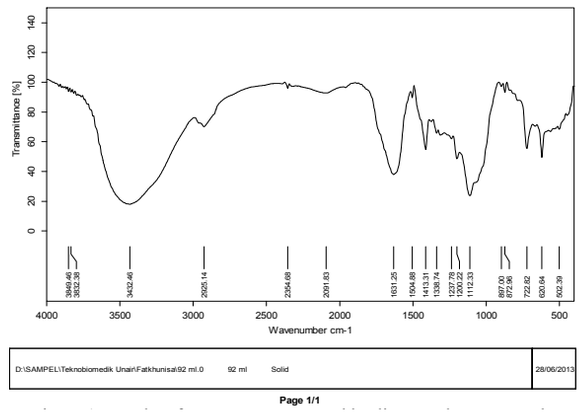

Figure 1. Results of FTIR Test on wound healing accelerator sample.

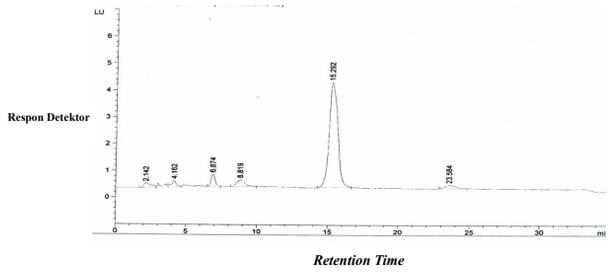

Figure 2. Spectrum of HPLC test results on *Achatina fulica* snail mucus.

Test Taxonomy Biological Characterization

Test taxonomy is the earliest process prior to the study as it aimed to get *Achatina fulica* snail species, according to the characteristics of the animal molluscs, based on accountable literature references.

Characterization of Fourier Transform Infra Red (FTIR)

Fourier Transform Infra Red (FTIR) test was used to determine the peak characteristics of functional groups described as transmittance curve (%) against wave number (cm^{-1}) on the material that has been made.

Characterization of High Performance Liquid Chromatography (HPLC)

To find out how much glycosaminoglican levels, one of the important growth factors activating proteins contained in the snail mucus wound closure as the main ingredient in accelerating wound healing.

Characterization of Anatomic Histopathology (AHP)

Wound healing and histology test were in vivo tests by observing the development of wound healing in mice (*Mus musculus*) macroscopically by the intensity of wound color, wound fluid, and wound type.[10]

Characterization of Antibacterial Test

This test used a disk diffusion method with *Staphylococcus aureus* and *E. coli* bacterial culture, whose inhibitory zone were analyzed. The qualitative disk diffusion test here used the colony units forming of 10^5 *E. coli* cultured on nutrient agar agent.

RESULTS AND DISCUSSION

Snails used in this research belonged to the phylum *Mollusca*, Classis *Gastropoda*, Order *Stylommatophora*, Familia *Achatinidae*, Genus *Achatina*, and species *Achatina fulica*. Snail shell length was 5.3 cm, it has smooth surface without ornament and has a color pattern of longitudinal stripes, alternating dark brown and yellowish white, the color lines are uneven edge, the dark brown part is wider than the white part. The shell is circular cone-shaped, the bottom coil is much larger than the other coil, and the concave coil has very clear boundaries. The shell appears strong and bold, but has very thin edge of the aperture, and the aperture is without cover.[6,7]

The result of Fourier Transform Infra Red (FTIR) test revealed a peak in the wave number 3750–3000 (showing O-H bond in alginate), 1900–1650 (showing C=O bond on the alginate), 1250–1050 (showing C-O bonds on the alginate) in accordance with existing references.[8]

High Performance Liquid Chromatography (HPLC) test showed that glycosaminoglycan content was found on the mucous of *Achatina fulica*. This finding is consistent with the literature, where the appearance of peak was at 165,477 in minutes.[9]

The final characteristic was the result of anatomic histopathology test conducted in mice (*Mus musculus*). The mice were given cut wound, and the healing process was observed through macroscopic test until the proliferative phase (around 13 days). Each sample group consisted of four mice. Parameters observed were the intensity of wound color, wound fluid, and wound type. The formed wounds contained liquid with a reddish color in each animal. The type of wound was open wound. On day 14, it could be seen that the composition of 4:2 was the most optimal composition in healing wounds since the reddish color in this group as a whole has faded, wound fluid in mice 1,3, 4 from a total of 4 mice had been absorbed by the wound healing accelerator membrane, whereas wounds in mice 1,2, and 4 had been completely closed. The next best composition was 4:3, then 4:1, and the last was a 4:0 or a group of negative samples. Through these observations, we observed that when the sample does not contain snail (*Achatina fulica*) mucus, the benefits of wound closing does not work et al.

The Antibacterial test results showed that from various concentrations used in tube dilution method, the concentration of 8% was the minimum dose of bacterial growth inhibition (MIC) and the concentration of 15% was the minimum concentration to kill the bacteria (MBC). Antibacterial test was only performed in a solution of 4:2 ratio since in macroscopic test the ratio had been proved as having most optimum wound healing.

Since the healing is known to be affected by tissue bacteria concentrations higher than 10^5 microorganisms per gram,[11] the use of dressing materials were able to reduce content of these microorganisms in surgical dermal wounds, as performed in this study, might be useful to avoid wound infection and, therefore, favor wound healing.[12] In addition, the release of angiogenesis-associated growth factors, such VEGF (vascular-endothelial growth factor) and PDGF (platelet-derived growth factor), after inflammatory chronification, is a key-step to the development of the granulation tissue during wound healing,[13] which could support our findings regarding to enhanced vascularization.

The composite of alginate, carboxymethyl cellulose (CMC) and *Achatina fulica* mucus can be produced in the early stages as an alternative wound dressings that have the potential of accelerating wound healing, absorbs excess exudate and prevent infection. From the above data it can be concluded that the most optimum composition of alginate and mucus are in 4:2 ratio. This comparison produces wound dressing that is still able to absorb exudate and optimally accelerate wound healing. In conclusion, we have demonstrated that the mucous secretion of *Achatina fulica* presents antibacterial properties. In addition, the use of dressing films based on this mucous secretion improved wound healing model.

CONCLUSION

The physical characteristics of accelerator wound healing membrane pore size are approximately 1,457-2,687 μm. The effect of Achatina fulica mucus as accelerator wound healing has been proved by the fact of faster healing process of wound compared with the group without the mucus.

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