The Application of Bromelain Enzyme on Pig Skin to be used as Surgery Training Model

Theddeus O.H Prasetyono
Indonesian Clinical Training and Education Center, Faculty of Medicine, Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta, theddeus.h@ui.ac.id

Christopher R. Andrian
Indonesian Clinical Training and Education Center, Faculty of Medicine, Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta

Elisabet L.A Kinanthi
Indonesian Clinical Training and Education Center, Faculty of Medicine, Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta

Siti R.K Putri
Indonesian Clinical Training and Education Center, Faculty of Medicine, Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta

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The Application of Bromelain Enzyme on Pigs Skin to be used as Surgery Training Model

Theoddeus OH Prasetyono, Christopher R Andrian, Elisabet LA Kinanthi, Siti RK Putri.

Indonesian Clinical Training and Education Center, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta.

Corresponding author: teddyohprasetyono@yahoo.com 
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Abstract

Introduction. Postmortem changes in pig skin made it difficult to be manipulated as a model for advanced technique in surgery training. The use of bromelain enzyme on these pigskins could be an alternative way to obtain the ideal surgery training model.

Method. Thirty-five pigskin blocks (10×15cm) was divided evenly into seven groups consisted of six groups of different bromelain enzyme treatment (4g/20min, 4g/40min, 8g/20min, 8g/40min, 12g/20min, 12g/40min) and one group of control. Then five chief-plastic surgery residents blindly assessed and scored each pigskin and compared its likeliness to the back of human skin. The scores were then analyzed with an interrater reliability test using SPSS 16.0.

Results. All five assessors unanimously chose the 8g/40min group as the most likeliness to the back of human skin. The highest score was obtained for piercing the needle into the pigskin (8.4 ± 0.54).

Conclusion. The appliance of 8 grams bromelain enzyme in 10×15cm pigskin blocks for 40 minutes was proven to be an ideal surgery training model comparing the back of human skin, especially for tangential excision.

Keywords: medical education, surgery training, model, pigskin, bromelain.

Introduction

The first utilization of pig as a surgery training model was back in 1961, where Straith et al. used pigs’ feet to practice suturing skin. Its use is widespread due to its fidelity to human skin, inexpensive, easy to obtain, and easy handling. Furthermore, based on its similar structures, such as thickness, collagen, lipid composition, and hair follicle content, pigskin opted to be the most suitable bench of the surgical training model. Although all the advantages, postmortem changes the tissues’ physical properties, making the texture and tenderness less realistic than human skin. The skin becomes challenging to be manipulated and penetrated with a needle or blade.

Bromelain is a mixture of proteolytic enzymes derives from the pineapple stem (Ananas comosus). It comprises glycosylated enzymes with different proteolytic activities. There are two main proteolytic active components of stem bromelain, which are F4 and F5, with molecular masses 24,397 kDa and 24,472 kDa, respectively. These two active components consist of 212 amino acids, with an optimum fraction of pH 4.0 to 4.5. The crude extract of bromelain shows its activity over pH 4.5 to 9.

In some literatures, bromelain enzyme has been studied for its usage in various sectors. In the health sector, the bromelain enzyme has been beneficial to prevent edema, promote the absorption of antibiotic drugs, and reduce tumor growth. Bromelain enzyme has also been used to debride burn wounds, and a promising option to treat bullous pemphigoid. In food technology, bromelain enzymes' proteolytic effect has been used as a meat tenderizer. The effect of tenderizing is described as the proteolysis, cleaving collagens in the meat's sarcollemma. Furthermore, it has been proven that it could increase the moisture and rigidity index of pork meat with bromelain compared to without tenderizer. In our experience in conducting surgical training for plastic surgery residents, we found that the immersion of pig skin into pineapple juice for two hours made the tissue more pliable, making it easier to manipulate the tissue as a surgery training model. However, we have not yet convinced ourselves whether the formulation works at best.

Method

This randomized control study found the most optimal dose and duration of bromelain enzyme application on pigskin to be used as a surgery training model. Pigskins, cut into 10×15 cm tissue block from the abdominal portion of a large white female breed at the age of nine months, weighted at least 90 kg. The pig was slaughtered in three days or less before and was frozen in -4° to -6° C until the study. There were six pigskin groups with different doses and bromelain duration; one group was without bromelain, referred to as control. Each group consisted of five pigskin blocks.

Bromelain Enzyme

The bromelain enzyme used was a commercial bromelain enzyme produced by PT. Bromelain Enzyme, Lampung (Gunung Sewu Group, Indonesia). The product was purified from a pineapple stem that had undergone several processes. The final product was a powder substance that had been proven to have the quality of 1300 CDU (Casein Digestion Unit).

Based on our previous trials and errors, we decided that the dose was divided into 4 g, 8 g, and 12 g of bromelain powder, with a duration of 20 minutes and 40 minutes each. Therefore, the groups consisted of the control group, 4g/20 minutes group, 4g/40 minutes group, 8g/20 minutes group, 8g/40 minutes group, 12g/20 minutes group, and 12g/40 minutes group.
minutes group. The powder was covered only on the skin’s surface, making sure that only the skin was in contact with the powder. This method was applied to ascertain that the subcutaneous layer structure was kept intact and visible to be differentiated.

Likelihood to the back of human skin
This study was measured with the assistance of evaluators. The evaluators were five chiefs of plastic surgery residents. They were selected at random with experience in suturing human skin at least once every week at the clinical workplace. After the treatment, the pigskins were washed with running water until free from traces of bromelain powder. Then, fixated onto a wooden plate with six nails on every corner of the skin. The skin was separated into two sections. The evaluators were instructed to do the tangential excision in the right section, resulting in approximately 2 cm in length and 1 cm in the split-thickness skin graft (STSG) width. The spherical excision was approximately 4 cm in length and 0.8 cm in width in the left section. The resulting defect was then sutured with two simple sutures on both sides of the edges using monofilament polypropylene 4.0; 3/8; 19 mm (Suripro, Covidien™). Due to the blunting needle and blade leading to bias, the suturing materials were renewed after four pigskins and a new blade for every pigskin.

All assessors were blinded about the treatment for the skin model. After all the tasks were completed on each pigskin, the evaluators had to fill a questionnaire. The questionnaire consisted of 6 questions with a scale of 1 to 10 on how likely the pigskin to the adult human back skin when the evaluators made incision; pierced the skin with the needle; undermined the tissue, felt the tension of the skin; made tangential excision; and the last question was about overall rate. The results were then evaluated with an inter-rater reliability test using SPSS 16.0. The assessors were also urged to give comments about the pigskin.

Results
All characteristics of the subjects are presented in table 1. In all treatment groups, the overall weight increased. The highest increase was found in the 8g/40 minutes, with an average increase of 21.2 (± 11.07). Moreover, there the pH decreased, where most of the treatment groups reached pH = 6.

Table 1. Subjects’ characteristics

| Characteristics                  | Control          | 4 grams          | 8 grams          | 12 grams         |
|----------------------------------|------------------|------------------|------------------|------------------|
|                                  | 20 minutes       | 40 minutes       | 20 minutes       | 40 minutes       | 20 minutes       | 40 minutes       |
| Total Subjects                   | 5                | 5                | 5                | 5                | 5                | 5                |
| Weight before treatment (grams)  | 245.6 (±29.18)   | 262.6 (±35.82)   | 259.00 (±33.52)  | 269.8 (±53.09)   | 335.2 (±18.63)   | 286.6 (±57.22)   | 308.4 (±33.53)   |
| Weight after treatment (grams)   | -                | 279.6 (±42.46)   | 273.4 (±31.44)   | 282.6 (±55.13)   | 356.4 (±22.12)   | 291.6 (±49.81)   | 328 (±34.40)     |
| Increase in weight               | -                | 17.0 (±0.49)     | 14.4 (±3.50)     | 12.8 (±2.71)     | 21.2 (±1.07)     | 5.0 (±10.5)      | 19.6 (±2.15)     |
| pH before treatment              | 7 (±0.63)        | 7.4 (±0.49)      | 7.0 (±0.75)      | 7.8 (±0.4)       | 7.6 (±0.49)      | 7.4 (±0.49)      |                |
| pH after treatment               | -                | 6.4 (±0.49)      | 6.0 (±0.5)       | 6.4 (±0.5)       | 6.0              | 6.0              | 6.0              |
| Skin thickness (mm)              | 14 (±2.0)        | 16 (±3.74)       | 13.0 (±4.0)      | 15.0 (±4.47)     | 16 (±5.83)       | 17.0 (±2.45)     | 12.0 (±4.0)      |
| Surface area (cm²)               | 165.95 (±15.87)  | 158.9 (±13.23)   | 162.6 (±9.14)    | 163.6 (±4.07)    | 157.3 (±9.12)    | 166.1 (±15.37)   | 166.1 (±15.37)   |

After the treatment of the bromelain enzyme, we found that there was no change in the color of the skin. However, the moisture and texture of the surface increased, making it less rigid. Few of the pigskins treated with bromelain had some of its epidermis layer peeled off; nonetheless, it was unnoticeable unless under magnification.

Likelihood to the back of human skin
The assessors measured the likeliness of the skin with a scale from 1 (very unlikely to the back of human skin) to 10 (very likely to the back of human skin) (Figure 3).

Making an incision. The incision on the pigskin has the highest likeliness to the back of human skin in the 8g/40 minutes groups with the average score of 7.8 (±1.30), and in the 12g/40 minutes groups with the average score of 7.8 (±1.78). The least average score is found in the control group, with an average score of 6.6 (±2.30). We found that the assessors’ agreement was inferior (κ = 0.03).

Undermining the tissue. All of the groups have relatively similar average scores in undermining the tissue (κ = 0.49). The highest average score is
found in the 8g/40 minutes group, 8.00 (±1.00), and the least average score comes from the control group and the 8g/20 minutes group with 7.2 (±1.64) and 7.2 (±1.48), respectively.

The likeliness of the pigskin to the back of human skin was based on the weight increased due to the tissue was holding more water from the enzyme traces on the pigskin. Therefore, we assume that the pigs bromelain enzyme using running water until there were no bromelain additional water. In this study, the pigskin was cleansed from the added water because of its physical properties.

WHC is defined as the tissue structure's ability to retain its water and is strongly correlated to the increase of Water Holding Capacity (WHC). The increase in the pigskin's weight after bromelain enzyme application ranged from 7.00(± 2.34) ranging from 3.00 to 9.00. Using the inter-rater reliability test, we found that the agreement of all the assessors was to be moderate (κ = 0.49).

The tension of the pigskin. The control group has the lowest likeliness to the back of human skin in terms of the pigskin's tension. The average score of 5.4 (±1.81) was compared to the 8g/40 group, with an average score of 8.2 (±0.84), making it the highest among all groups. The other treatment groups have similar results ranging from 6.4 to 7.8 (κ = 0.49).

The resistant properties of the pig skin20 were measured with the score given by the evaluators when they were making an incision, piercing the needle, and making tangential excision. When making an incision, the evaluators’ average score piercing the suturing needle in the 8g/40 minutes group was higher compared to the control group. Less resistance of the bromelain-enzyme-applied skin caused by breakage of the collagen network in the skin by the enzyme increased the tenderness and softness of the pigskin.10,11,12,22

Overall likeness. Overall, all evaluators gave 8g/40 minutes groups the highest score with an average of 7.8 (± 0.84), and the least score for the control group with an average of 5.8 (±1.09). The reliability test for the overall score was moderate (κ = 0.49)

The likelihood of the pigskin to the back of human skin was the basis on the evaluators' experience. Some of the evaluators' comments were "the pigskin is too lenient" or "it is too rigid," as the reason for giving bad scores. Some of the evaluators also felt that the skin was too fragile in the 12g/40 minutes group for tangential excision.

Even though the reliability tests were varied in all aspects, the highest average of the pigskin's likeliness to back human skin was found in the 8g/40 minutes group in all aspects that were evaluated. The average score of the 8g/40 minutes group from all aspects evaluated ranged from 7.8-8.4, while the control group was ranging from 2.6-7.2. A significant correlation was also found in the 8g/40 minutes group in the tangential excision with an average score of 8.0 compared to the control group with an average score of 2.6 (p <0.05).

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Conclusion

The 10x15 cm block of pigskin with the surface that has already been applied by 8 grams of bromelain enzyme (powder, 1300 CDU) for 40 minutes has the highest likeliness to the back of human skin other doses and durations, especially regarding tangential excision. Although it was assessed to be poor for making an incision, the model was moderately suitable for other procedures. Finally, the pigskin applied with bromelain is more suitable for the surgery training model than raw pigskin.

Disclosure

Authors disclosed no conflict of interest
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