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Development of polyurethane foam dressing containing silver and asiaticoside for healing of dermal wound

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ABSTRACT

Polyurethane foam dressings for dermal wounds were formulated with natural polyols in order to improve the foam characteristics and the release of 2 active agents, silver and asiaticoside (AS) as an antimicrobial agent and an herbal wound healing agent, respectively. The foam was instantly formed by interaction of polyols and diisocyanate. Hydroxypropyl methylcellulose, chitosan and sodium alginate were individually mixed with the main polyols, polypropylene glycol, in the formulation while the active components were impregnated into the obtained foam dressing sheets. Although the type and amount of the natural polyols slightly affected the pore size, water sorption-desorption profile and compression strength of the obtained foam sheets, a prominent effect was found in the release of both active components. Among natural polyols formulations, foam sheets with alginate showed the highest silver and AS release. Non-cytotoxicity of these foam sheets to human fibroblast cells was confirmed. Antimicrobial testing on four bacteria strains showed that 1 mg/cm² silver in formulations with 6% of natural polyols and without natural polyols had sufficient content of the silver release with comparable inhibition zone and significantly larger zone than other formulations. In pig study, the foam dressing with 6% alginate, 1 mg/cm² silver and 5% AS could improve wound healing in both the percentage of the wound closure and histological parameters of the dermal wound without any dermatologic reactions. In conclusion, this innovative foam dressing had potential to be a good candidate for wound treatment.

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1. Introduction

The primary objectives of wound care are rapid wound closure, minimal complications and less chance of hypertrophic scar formation. Dermal wound or partial thickness wound has pathology in both epidermis and dermis layers, and is characterized by blebs, pink-red color, moist and pain. Because of available cells, this wound type can be self-healed under appropriate treatment especially with wound dressing that possesses tissue regeneration and healing properties. There are many factors to be considered to develop advanced wound dressing. Ideally, wound dressing should provide the effectiveness in exudate absorption, retaining hydration, prevention of micro-organism contamination and avoiding dressing related trauma [1].

Due to thicker than other wound dressings, the foam dressing provides a protective cushioning effect over the wound. Not only prevent exudates pooling and periwound maceration, it also keeps hydration so that the appropriate moisture environment will promote the epithelialization and healing through the encouragement of the migration of cells [1,2]. Polyurethane (PU) foam was generally synthesized by the reaction of polyols with isocyanate [3]. The open cells and linked porosity in foam structure were caused by two reactions; gelling and blowing. The carbon dioxide gas from the blowing reaction expanded the foam while simultaneously the gelling reaction would produce the urethane linkage which increased the foam strength. Concerning the water sorption and moisture protection, hydrophilic natural polymers such as cellulose and polysaccharides were for the first time to be incorporated in foam formulation.

Apart from possess water-favorable property, hydroxypropyl methylcellulose (HPMC, H), chitosan (CLMW, C) and sodium alginate (Alg, A) are investigated due to low cost, commercial availability, and excellent biocompatibility. To date, carboxymethylcellulose (CMC) has been incorporated in various commercial wound products such as IntraSite™ Gel, GranuGel™, and Aquatech™ Ag, but not HPMC. Only investigation on HPMC based hydrogel were reported that over 95% wound closure and collagen deposition in a few weeks after application of ofloxacin-loaded HPMC hydrogels [4]. In rat study, HPMC/chitosan gel loaded with simvastatin showed significant increase in wound closure [5]. Chitosan has markedly wound healing properties due to its antimicrobial effect, hemostasis stimulation, and acceleration of tissue regeneration [6-8]. Numerous studies have reported on chitosan containing formulations for wound healing [5,6,9]. Sodium alginate, an anionic polysaccharide has outstanding in hydrophilic property. The alginate is commonly prepared as alginate hydrogel dressing which turns to gel after water sorption such as Algisite® M and Kaltostat™ [10]. The alginate dressing preserved a moist environment around the wound to promote wound healing including the histological results of facilitation on wound healing from neomycin sulfate-loaded alginate hydrogel dressing compared to the commercial product [11]. Thus the incorporation of these hydrophilic polymers in PU foam was expected to intensely improve foam dressing properties.

To reduce systemic antibiotic resistance, silver (Ag) in dressings locally improve wound treatment especially in chronic and infected prone wounds. This metal has 4 possible bacteriostatic/bactericidal mechanisms [12,13], binding with bacterial cell wall leading to cell membrane disruption, binding microbial DNA effect thus inhibiting cell division, generating reactive oxygen species and free radicals and blocking the mitochondrial respiratory function by inhibiting respiratory chain enzymes. This agent is a valuable candidate for wound management with low incidence resistance [12,14]. Moreover, numerous evidences have proved the efficacy of silver dressings in both in vitro and clinical trials and the removal of the bioburden in burn and open wound has been indicated [15-17].

Asiatioside (AS), an active herbal compound from Centella asiatica, has been confirmed its potency in wound healing [18-20]. The solution containing AS increased the hydroxyproline content, tensile strength, collagen content and epithelialization resulting in facilitating the healing of diabetic wounds [21]. In an in vivo animal burn model, AS showed positive effects on the proliferation and cell growth of wound healing by stimulating the collagen synthesis and reducing the wound’s oxidative stress [22,23]. Faster and better maturation of collagen was reported in the extract-treated wounds whereas Smad 3 and 4 signaling in collagen synthesis was induced [24,25]. Moreover, the Centella asiatica extracts were listed in the Indian Pharmacopoeia as wound healing agent [26].

Owing to similar skin structure to the human such as the process of reepithelialization, vasculization and percentage amount of collagen and elastic fiber in the extracellular matrix [27,28], pig model has generally been used to investigate the in vivo effectiveness of wound treatment. The dermis thickness of pig is about 1400–2000 μm compared to human which is about 2000–3000 μm while the rat and mouse skin are thinner (1000–2000 and 200–600 μm, respectively) [29]. It also has sparse hair (10–20 hairs/cm²) rather than fur which differs from rodent and rabbit (289 and 658 hairs/cm², respectively) [30]. There is only one sebaceous gland per hair follicle in pigs and humans whereas two opposite glands per each hair follicle are in rodents [31].

Thus the aim of this study was to investigate the influence of three natural polyols on the physical and mechanical behavior of PU foam dressings especially the effectiveness in exudate absorption and retaining hydration. The content of silver and AS, their releasing profiles and antimicrobial activity were also determined in Part I of this investigation to select the most appropriate formulation. Then, the optimized dressing would then be tested for the wound healing efficacy and safety in animal models in Part II study.

2. Materials and methods

2.1. Materials

The materials used for the study comprised polypropylene glycol (PPG, MW 3000), silicone copolymer surfactant (Dabco DC5810), amine catalyst (Dabco 33-LV), tin catalyst (T9), and toluene diisocyanate (TDI) that were obtained from the Air Products and Chemicals Company Limited (Pennsylvania,
USA). Methylene chloride came from Fisher Scientific (New Hampshire, USA). Other hydrophilic polyols used included hydroxypropyl methylcellulose (HPMC, Methocel® ES, Col- orcon Asia Pacific Pte. Ltd., Singapore), chitosan (CLMW, Sigma-Aldrich Chemical Company, Missouri, USA), and sodium alginate (Alg, Acros Organics, Geel, Belgium). Silver nanoparticles were bought from the Guangzhou Hongwu Material Technology Company Limited (Guangzhou, China; Batch No. HW-P160819). Asianoside powder (95% purity) was bought from the Xian Lyphar Biotech Company Limited (Shaanxi, China; Batch No. LYPH150927). All other chemicals were AR grades and used as received.

2.2. Preparation and evaluation of foam dressing properties (Part I)

2.2.1. Preparation and characterization of PU foam dressing

2.2.1.1. Preparation. PU foam was produced by the reaction of polyols with diisocyanate [3]. Briefly, polypropylene glycol (PPG) 100 g and 4%–6% of a hydrophilic natural polyols; HPMC (H4, H6), CLMW (C4, C6) or Alg (A4, A6), 2.0 g of deionized water, 1.5 g of silicone copolymer surfactant, 3.0 g of methylene chloride, and 0.2 g of amine catalyst were mixed and vigorously stirred by an impeller at 500 rpm for 2 min before being cooled to 20 °C. Tin catalyst was added to the mixture, stirred at 1900 rpm for one min followed by adding 31.71 g of cooled toluene diisocyanate (TDI) and stirring for a few seconds. The load of foam was immediately created after the mixture was poured in a mold at 25–30 °C and then cured for 72 h. Foam sheets were sliced by a foam cutting machine (Foam vertical cutting machine model IS-M, Albrecht Bäumer GmbH and Company KG, Freudenberg, Germany) to obtain a thickness of 6.0 ± 0.5 mm. The blank foam sheet without natural polyols (Bl) was also prepared.

2.2.1.2. Characterization. Microscopic appearance and pore size: Prior to the investigation, samples were cut with a blade and attached on the stub then coated with gold. Scanning electron micrographs of samples were taken at 10 kV, 15x magnification (JSM-7610F, JEOL, Tokyo, Japan), and the pore sizes from the top view were calculated [32] from approximately 300 pores using the Image J program (NIH, USA).

Density: The foams were measured in width, length, and thickness using a Vernier caliper (Mitutoyo digimatic caliper series 500, Tokyo, Japan) in mm. Then, the samples were weighed and recorded in grams. The density was calculated and reported as g/cm³ unit [33].

Water absorption and %weight loss study: Foam sheets were dried in a desiccator for 24 h before the initial weighing (Wf) then held in stainless steel mesh tea ball apparatus prior to submerging in 120 ml of distilled water. After equilibrated at 37 °C for 48 h, the samples were then taken out of the water, suspended for 10 s for free drainage, absorbed excess water with paper and reweighed again to get Wf. Subsequently, the samples were kept in an incubator at 50% ± 5%RH, 37 ± 1 °C for 48 h, the samples were reweighed. Weight loss was the weight that was lost from the absorption and desorption process and was calculated from the difference in weight between Wi and Wd. Five samples per formulation were evaluated. The percentages of the absorption and weight loss could be calculated from absorption %= (Wf – Wi)/Wi × 100 and weight loss %= (Wf – Wd)/Wf × 100 [34].

Foam degradation test: The study was applied from the enzyme degradation test [35]. The dry sample was weighed (W0) and then completely suspended in 7 ml of phosphate-buffered saline pH 7.4 with or without lysozyme (1.6 µg/ml) in the 6-well plate and incubated at 37 °C. The solution was refreshed daily. After 48 h, the sample was removed, washed with distilled water, and dried at 40 °C for 48 h. The dry weight was measured (Wi). The percentage of degradation = (W0 – Wi)/W0 × 100%.

Compression test: The study was applied from ASTM D3574 [36] using a Universal Testing Machine (UTM) (Shimadzu, model EZ-S 500N, Osaka, Japan). The initial thickness of the sample was determined prior to placing between the horizontal plates of a compression device. The samples were compressed to 75% of their original thickness with a speed of 2 mm/min after that the samples were removed. The compressive strength values were reported at 50% of the strain. Five specimens per sample were tested. The value was reported as a mean value of those observed.

2.2.2. Preparation and determination of active compounds contents and their releasing profiles

2.2.2.1. Preparation. Active ingredients were loaded into the foam sheets of 10 × 10 cm² by an absorption process. Silver nanoparticles of 0.4, 0.6, 0.8 and 1.0 mg/cm² (0.4–1.0Ag) were homogeneously dispersed in deionized water of not more than optimal water absorption volume of the foam whereas asianoside powder was also incorporated at 5% in selected dispersions. After absorption, the foam sheets were oven dried at 40 °C for 48 h.

2.2.2.2. Determination of active compounds contents and their releasing profiles

Silver content

This experiment was adapted from Kulthong et al. [37]. The sample was added with 1 ml of 50% (v/v) of nitric acid and boiled in a water bath (Model B 22, Memmert GmBH + Company KG, Schwabach, Germany) at 70 °C for 2 h. Then, 0.5 ml of the acid solution was pipetted to 2 ml of distilled water. The solution was centrifuged at 3000 rpm for 5 min. The 1 ml supernatant was pipetted into 4 ml of purified water. The amount of silver ion was determined by a flame atomic absorption spectrophotometer (AAS, Varian model AA280FS, California, USA) at the wavelength and lamp current of 328.1 nm and 3 mA, respectively. The flame type was air/acetylene with air flow and acetylene flow of 13.20 and 1.8 l/min, respectively. The standard concentration was prepared from a standard silver solution (Merck KGAa, Damstadt, Germany).

Asiaticoside (AS) content

The cubed foam was suspended in 5 ml of methanol. The suspension was shaken in a water bath at 30 °C for one h. The process was repeated in new medium. Both volume of medium was then filtered through a 0.45 µm membrane filter prior to injection. The AS content was determined following the method by Hengsawas et al. [38] using HPLC with a UV detector at 220 nm (Shimadzu model LC-20AB, Shimadzu.
Scientific Instruments, Kyoto, Japan, detector model SPD-20A) and HALO-5® (C18) column (250 mm × 4.6 mm), 5 μm (Advance Materials Technology, USA). The injection volume was 20 μl at a flow rate of 1 ml/min. The mobile phase was water-acetonitrile with linear gradient conditions of water 70%, 0%, 70% and 70% of water in pump A, and 30%, 100%, 30%, 30% acetonitrile in pump B at the time intervals of 0, 12, 15 and 30 min, respectively.

Releasing profiles of active compounds

A release study was applied using the static Franz diffusion cells method [39]. Purified water and PBS pH 7.4 with 10% of methanol were used as receptor media for the content determination of silver and AS, respectively. The receptor compartment of each medium was maintained at 37 °C and magnetically stirred. A round shape dressing sample was placed between the donor and the receptor compartment. At various time intervals, the solutions of appropriate volume were sampled from the receptors for determination of the amount of silver and AS released, respectively and replaced with the same volume of fresh solution to maintain the fluid level. The 4 ml of silver sampling solution was mixed with 1 ml of 25% (v/v) nitric acid to dissolve the silver nanoparticles. The contents of silver and AS were quantified by using AAS and HPLC as aforementioned, respectively.

2.2.3. Antibacterial test

An agar diffusion method was performed in this study with some modifications [40] using four bacterial strains commonly found in trauma wounds; Staphylococcus aureus (ATCC 6538), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) [41]. The bacteria were grown in a culture slant for overnight incubation. The bacterial culture was suspended in broth, and the turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standards (1.5 × 108 cfu/ml). The broth of 100 μl was used to streak on Muller-Hinton agar plates in three directions to form a confluent lawn. The sample of the ascertainment size was aseptically applied to the center of each lawn. The plates were left in the incubator at 37 °C for 24 h. The diameter of the clear zone surrounding the test dressing was measured for the zone of growth inhibition using a Vernier caliper and recorded in millimeters (mm). All zones of the inhibition (ZOI) were reported as a mean and standard deviation from three independent experiments.

2.2.4. Cytotoxicity test

The human fibroblasts (ATCC® CRL-2522™) were cultured in complete growth medium which composed of 10% of fetal bovine serum, 1% of antibiotic-antimycotic agent which contained amphotericin B, penicillin and streptomycin and Dulbecco’s Modified Eagle’s Medium (DMEM) which contained a saline solution, amino acids, 25 mM of D-glucose, and 1 mM of sodium pyruvate. The cell culture grew in a 37 °C and 5% humidified CO2 incubator.

Study of the viability of the cells in the presence of foam samples was modified from Burd et al. [42] whereby the fibroblasts were planted in a complete growth medium on 24 well plates at a density of 5 × 103/well. The plate was incubated in 5% of CO2 at 37 °C for 72 h to obtain 70% of the cells’ confluences. The complete growth medium was added into each well for 300 μl. At that time, the 1 × 1 cm2 dressing samples soaked with 200 μl of phosphate buffer solution were added to the culture well. The control was 500 μl of complete growth media solution. After 24 h of incubation, the dressing and medium were removed. The cell viability was determined by an MTT assay. The absorbance was measured at 560 nm in triplicate (100 μl in three wells of 96 well plates), using a microplate reader (Perkin Elmer Victor3™ Model 1420-050, Massachusetts, USA). The number of viable cells is correlated with a mitochondrial activity which reflected by the conversion of the tetrazolium salt MTT into formazan crystal by the mitochondrial dehydrogenase enzyme. The percentage of the cell viability was calculated from the following; cell viability% = (Absorbance of surviving fibroblast of samples / Absorbance of surviving fibroblast of control) × 100.

2.3. Efficacy and safety in the animal model

2.3.1. Skin irritation test of selected foam dressing on rabbits

This experiment was approved by the Institutional Animal Care and Use Committee of Thailand Institute of Scientific and Technological Research (TISTR) (Protocol No.TS-59,001) according to the OECD Guidelines (2015) [43]. Three rabbits were housed individually within approximately 20 ± 3 °C and 50% ± 10% RH, for adaptation to minimize stress and physiologic alteration before the experiment. Conventional laboratory diets with drinking water were provided ad libitum. Twenty-four hours before the test, the fur on the dorsal area was removed and avoided abrading the skin. The 4.0 cm2 of the tested dressing was soaked with 0.5 ml of normal saline solution before being applied on the skin. Then, it was covered with a sterile gauze patch and held in place with adhesive tape. The skin area, which was applied with the dressing, was called the study group. On the other side, the control group was applied with a gauze patch and adhesive dressing. The experiment was left for 4 h then removed and gently cleaned with cotton balls soaked with normal saline solution. The redness and swelling response grading were between 0-4 points where 0 was defined as no serious skin reaction and 4 was a serious reaction. After dressing removal, the evaluations were recorded at 1, 24, 48 and 72 h, respectively. If there was any skin reaction, a confirmation test on two more animals would be taken into consideration.

2.3.2. Efficacy and safety of prepared foam sheets on pigs

Five domestic farm pigs with an average weight of 20–25 kg were allocated for the study after authorization by Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC), Mahidol University (Protocol No. MUVS-2016-09-34). The experiment followed the guide for the care and use of laboratory animals (NRC 2011) and the guide for the care and use of agricultural animals in research and teaching (FASS 2010). After seven acclimatization days, the animals were made to fast 12 h before surgery. All animals were intramuscularly injected with tiletamine-zolazepam to induce anesthesia and maintained by administering isoﬂurane in 100% oxygen. In each pig, the deep partial thickness of the excision wounds (area about 225 mm3) were created along the markings us-
ing toothed forceps, a surgical blade and pointed scissors. The total number of experimental wounds was 50, with 10 per animal. All wounds were cleaned with a sterile normal saline solution following the program for each group. These created wounds were randomly assigned into five groups of treatments as follows: Group I (comparative group I) was treated with a commercial PU foam dressing, Group II (comparative group II) was treated with a commercial silver coated PU foam dressing, Group III-V (study group I-III) was treated with PU foam dressing containing at least 2 components following; 1) natural polyols, 2) silver and/or 3) asiaticoside. These 3 study groups would be selected from releasing profiles. All of the wounds were applied to the dressing, then covered with sterile gauze, and changed every one-two days. During the observation of the wound healing, the wound area was periodically recorded and calculated (in cm²) at 0, 4, 7, 14 and 21 d A digital camera (SONY, model DSC TX9, Sony Company Limited, Japan) was used to collect the wound appearance with 10 cm above the wound. During this study, the animals were routinely checked for food and water consumption and mention.

2.3.2.1. Histological evaluation Punch biopsies were taken at two time points: 7 and 14 d post-wounding at the wound’s edge. The tissue was taken and fixed in 10% of buffered formalin solution. Each specimen was embed-
ed in a paraffin block and stained with hematoxylin-eosin and Masson’s trichrome method. The tissue from the normal skin would be collected in order to compare with the tissue from the wounds. After that, the tissue was examined histologically under a light microscope (Nikon Eclipse E200, Nikon Instruments, Tokyo, Japan). The histologic examinations were modified from the studies of Abramov et al. and Karayannopoulou et al. [44,45] and detected per high power field (HPF) at a magnification of 400. For the comparison to normal skin, the epithelium cell layer, amounts of the inflammatory cells and fibro-
lasts were counted and scored using 4 scales: 0 = normal, 1 = mild increase, 2 = moderate increase and 3 = marked increase. The new capillaries formation was assigned an angiogenesis score of 0 = < 3 new vessels, 1 = 3–10 new vessels, 2 = 11–30 new vessels and 3 = ≥ 31 new vessels detected per HPF.

2.4. Statistical analysis

The mean and standard deviation of the groups were calculated for each data set. The differences in all quantitative data; such as compression test, drug contents, the zone of inhibition, wound area, the day of epithelialization and histological score between group were compared using one-way ANOVA, and the releasing profiles were compared using repeated measured ANOVA. The comparison of histological score between day 7 and 14 within group was perform using paired t-test. The statistical significance was considered less than 0.05. All statistical data analyses were performed using SPSS 22.0 (SPSS Company Limited, Bangkok, Thailand). The qualitative data; such as dermatologic effect were reported as descriptive information.

3. Results and discussion

3.1. Preparation and evaluation of foam dressing properties (Part I)

3.1.1. Preparation and characterization of PU foam dressing

3.1.1. Preparation The obtained foam dressings were white, soft, flexible and also immediately recovered after compression. Foams with chitosan had a little yellowish color.

3.1.1.2. Characterization Microscopic appearance and pore size: The morphology observed by SEM showed several round-shaped and interconnected pores within the foam (Fig. 1). The average pore sizes from all groups were similar to commercial wound dressings [32]. BI foam seemed to provide little larger pore size compare to natural polyols formulations (P > 0.05). The average pore size of natural polyols foams was in a range of 228–262 μm with no significant differences (P > 0.05). The porosity slightly decreased with increasing the polymer solution concentration. This might cause by the opportunity of natural polyols could react with disiocyanate more than foam without natural polyols [46]. The polymerization might comp-
act the foam structure. Another reason was viscosity in the formulation. The natural polyols powder which was added in suspension could increase viscous and hinder gas creation [47,48].

Density: The calculated density of BI was less than foam with natural polyols (Table 1). The concentration of natural polyols might increase foam density, however, there were no significant results (P > 0.05). The density of foam might be an inverse relationship with pore size. The porosity of foam was expressed by P% = (1 - Dfoam/Dpolymer) × 100, where Dfoam and Dpolymer were density of foam and actual density of the polymer, respectively [49]. From this equation, it could be inferred that the density of foam would increase when the porosity de-
creased.

Water absorption and %weight loss study: The natural poly-
ols could facilitate water absorption compared to foam with-
out natural polyols (Table 1). Their percent water absorption at 48 h results were higher than BI group but no significant differ-
cences were noted (P > 0.05). The polyol groups which were left from polymerization would react to water via hydrogen bond-
ing. According to their structures, these three natural poly-
mers had some different substituted groups; OH and OCH₃ group from HPMC, a COO-Na+ group from Alg and NH₂ group from CLMW. The foam with HPMC seemed to have water sorp-
tion higher than Alg foam. However, it has been reported that the tablets containing Alg could swell more rapidly than those of HPMC [50]. The obtained foam with Alg might consist of some unreacted Alg which could swell and dissolve easily. Thus it could not sustain much water within their network structure [51].

The foam containing Alg could absorb water more than CLMW foam in both two concentrations. The water sorption ability of foam dressings containing natural polyols might be explained from previous studies. The number of water molecules absorbed per repeating unit in the amorphous phase can be ranked as follows: alginate > chitosan [52]. The water sorption first occurred on polymer sites. The chitosan
could interact with two water molecules per repeating unit at NH₂ group while four molecules are bound per repeating unit at COO⁻ group in Alg. HPMC foam also presented good water sorption property comparable to Alg foams. This might be explained by functional groups contained in polymers. The degree of substitution and viscosity grade are also involved in polymer hydration and drug release. Although cellulose generally could bind 2 water molecules per glucopyranosyl unit, while the HPMC E5 could bind 6 water molecules [53,54]. The hydration of HPMC also presented the net exothermic value more than the other cellulose [53]. The stronger binding with water of functional groups might lead to increase absorption capacity.

Another factor related to water absorption is porosity. Small pore size could prevent the water leak out during handling. Thus larger average pore size of B1 foam could retain less water than foam with natural polylols. Among natural polylols groups, the H6 had the smallest pore size while C4 showed the largest. At 48 h, the water sorption of HPMC was the highest while the lowest was from CLMW.

The percent weight loss of natural polylols foams was higher than foam without natural polylols. The hydrophilic groups in natural polymers could interact with water and then solubilized. The polarity of organic compounds could be ranked: acid > alcohol > amine groups. This might reflect to their percent weight loss that Alg > HPMC > CLMW foams. The hydrolysis effect of Alg foam seemed to be higher than that of other foams because Alg contained a salt form of carboxylic groups (COONa) which presented the strongest hydrophilicity. The hydroxyl groups in HPMC could also interact with water molecules but showed lower percent compared to Alg. While CLMW had percent weight loss comparable to B1 possibly caused by the pKa value of 6.5 of CLMW. The solubility of chitosan depends on the protonation of free amino group that it could solubile in acidic solution and also hardly solubilize in deionized water which had pH nearly 7.0.

**Foam degradation test:** The percentage of the weight loss of various foam dressings in the solution of lysozyme which generally found in wounds and in the phosphate buffer pH 7.4 compared at 48 h are shown in **Table 1**. It could be seen that adding natural polylols seemed to increase the weight loss of the foam dressing% especially in formulation with Alg except in formulations with CLMW. Moreover, increasing the amount of polylols would increase the weight loss due to polymer solubilization. In addition, there was no significant difference in the weight loss in the lysozyme and buffer solutions (P > 0.05) indicating that lysozyme had no effect on the dressing integrity. Minute residue could be found in the wound due to

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**Table 1 – The physical properties and integrity of foam dressing.**

|          | Mean pore size (x 10⁻³ mm) | Density (g/cm³) | Water absorption 48 h (%) | Weight loss 48 h (%) | Enzyme degrade 48 h (%) | PBS degrade 48 h (%) | Mechanical strength (x 10⁻³ MPa) |
|----------|-----------------------------|-----------------|----------------------------|----------------------|------------------------|----------------------|-------------------------------|
| B1       | 317.65 ± 264.76             | 41.09 ± 1.09    | 1104.29 ± 142.13           | 0.15 ± 0.34          | 0.72 ± 0.82            | 1.14 ± 1.05          | 4.60 ± 1.05                   |
| H4       | 243.79 ± 238.99             | 44.54 ± 0.94    | 1353.05 ± 490.75           | 1.68 ± 0.41          | 2.19 ± 1.11            | 1.22 ± 1.43          | 5.61 ± 0.65                   |
| H6       | 228.91 ± 226.36             | 44.81 ± 3.07    | 1515.25 ± 520.39           | 2.51 ± 1.94          | 3.19 ± 1.11            | 1.50 ± 1.00          | 5.42 ± 0.79                   |
| C4       | 262.37 ± 238.31             | 41.25 ± 1.63    | 1231.88 ± 373.05           | 0.06 ± 0.34          | 0.49 ± 0.67            | 0.29 ± 0.87          | 5.08 ± 1.05                   |
| C6       | 238.93 ± 225.15             | 41.20 ± 1.46    | 1253.47 ± 255.97           | 0.07 ± 0.11          | 0.58 ± 1.46            | 0.43 ± 0.64          | 5.18 ± 1.14                   |
| A4       | 239.43 ± 267.74             | 41.94 ± 1.95    | 1301.83 ± 358.97           | 2.86 ± 0.68          | 2.61 ± 1.03            | 2.16 ± 1.57          | 4.99 ± 0.66                   |
| A6       | 236.35 ± 238.52             | 42.28 ± 3.57    | 1512.69 ± 597.99           | 3.70 ± 0.45          | 4.34 ± 0.79            | 4.37 ± 1.05          | 5.29 ± 0.29                   |
the degradation of the natural polyols, especially from A6 formulation.

Compression test: Adding natural polyols seemed to increase the strength. The HPMC formulation had a similar strength to A6. At the same compressive distance, there were no significant differences between the groups (P = 0.593). The compressive strength was associated with pore size and density [55]. The higher amount of powder in the mixture such in formulation with 4% and 6% of natural polyols would lead to higher viscosity and also increase foam density and also increase the mechanical strength of the final foam. The compressive strength could explain the response of the foam dressing while it experienced a compressive load. The force would decrease the pore sizes.

3.1.2. Determination of the active compounds contents and their releasing profiles

3.1.2.1. Active compound contents

Silver content

The silver contents after preparation were in a range of 92.50%—94.50% of theoretical amount. Foam with Alg seemed to have the highest silver content while foam with chitosan showed the lowest (94.37% ± 8.61% and 92.50% ± 9.40%, respectively). However, there were no significant differences between the silver amounts among 4 formulations (P > 0.05). Some silver losses in all groups was due to some residue was found in container after absorption process. The agglomeration might occurred in the concentrated silver suspension which hardly solubilized and easily fallen in the bottom before the content determination.

Asiaticoside (AS) content

The AS content was 94.0%—96.0% after impregnation and no statistical differences between groups (95.47% ± 8.81%, 95.29% ± 8.35%, 95.21% ± 10.64% and 94.42% ± 10.90% for foam dressing without natural polyols impregnated with silver and AS (Bl-1Ag-AS), foam dressing with 6% HPMC impregnated with silver and AS (H6-1Ag-AS), foam dressing with 6% CLMW impregnated with silver and AS (C6-1Ag-AS) and foam dressing with 6% Alg impregnated with silver and AS (A6-1Ag-AS), respectively, P > 0.05). Apart from the residue after foam absorption, AS might degrade from drying process [56]. Heat condition resulted in a decrease of total triterpene glycosides.

3.1.2.2. Releasing of the active compounds

Silver releasing profiles

The releasing profiles of Bl, HPMC (H4 and H6), CLMW (C4 and C6) and Alg (A4 and A6) are shown in Fig. 2. Concentrations of both silver and natural polyols affected the silver releasing profiles. Higher amount of Ag in dressing provided the higher amount of Ag release. The burst release of silver after the water contact was due to some deposition on the surface. The releasing from pore and the agglomeration of small
silver nanoparticles when releasing from foam dressing would prolong the release. The size of released silver nanoparticles was shifted to a slightly larger population [57]. The silver releasing mechanism involved two phases; the initial silver dissolution and then agglomeration that the latter reduced the surface area leading to decrease the amount and also prolong the rate of releasing profiles. In addition, the silver nanoparticles might show low releasing profiles in deionized water. The property of a vehicle affected the active compound releasing. A lower pH of a vehicle would provide the silver dissolution more than neutral pH [58,59]. Although low amount of silver releasing profiles, there was a concept of silver containing in suprasorbent wound dressing. In order to avoid periwound maceration, the dressing should absorb excess exudate. In this step, some bacteria in exudate were absorbed and killed by silver inside the dressing [60].

The higher concentration of polyols provided higher releasing profiles. The 6% of natural polyols could facilitate silver release more than 4% In the HPMC group, H6-1.0Ag > H4-1.0Ag > H6-0.4Ag > H4-0.4Ag. In the CLMW group, C6-1.0Ag > C4-1.0Ag > C6-0.4Ag > C4-0.4Ag. In the Alg group, A6-1.0Ag > A4-1.0Ag > A6-0.4Ag. Among groups containing 1.0 mg/cm², A6 gave the highest release, and higher than C4 and C6 (P = 0.013 and 0.021, respectively). The releasing profiles of silver from the Bl group were quite instant compared to the natural polyols group due to larger pore size which allowed water and drug to pass through easily. Moreover, the Bl foam had not hydrophilic functional groups thus there was no swelling effect to hinder permeation. Among three polyols, COONa from Alg foam was the strongest hydrophilic group. The erosion effect of Alg foam could increase the silver releasing profiles which could be confirmed by weight loss%. The OH group also presented water-favorable property. The releasing profiles of HPMC foam were comparable to Alg foam. The CLMW foam seemed to provide low releasing profiles. This might cause by the polarity of amine group which was weaker than COONa and OH group. From this study, 6% of the natural polyols and 1 mg/cm² of the silver concentration was selected for the next experiments.

Fig. 3 – Asiaticoside releasing profiles of foam without natural polyols [Bl-1Ag-AS], foams with 6% of HPMC (H6-1Ag-AS), CLMW (C6-1Ag-AS) and Alg (A6-1Ag-AS) impregnated with 1 mg/cm² silver and 5% asiaticoside.

Asiaticoside releasing profiles

It was clearly shown that the Bl-1Ag-AS rapidly released the highest amount of AS, and the release was constant after 8 h (P < 0.05), followed by A6-1Ag-AS while C6-1Ag-AS was the lowest (Fig. 3). Similar to the silver release, AS from Bl-1Ag-AS was quite rapidly released then become gradually decrease. The releasing profiles of natural polyols foams were similar to foam without natural polyols. Similar to silver release profiles, AS on surface could burst release following by diffusion of drug through pores and channels. The larger porosity from foam without natural polyols provided faster rate of AS releasing profiles from foam with natural polyols. In addition, the latter foams could absorb water and swell more than the former foam that swelling effect and gelling behavior of polymer might retard the drug releasing in the hydration process [61,62]. The erosion effect could be found in hydration confirmed by percent weight loss [63] that higher weight loss% from foam with Alg related to increase the release of the drug. Although formulations with H6-1Ag-AS and C6-1Ag-AS had a comparable low release, the latter foam gradually released the AS even after 48 h while the former reached the plateau after 24 h. The less swelling and solubility of chitosan in buffer solution could delay the releasing profiles. Moreover, the positive charge of amine group rich in CLMW might attract

### Table 2 – Comparison of the inhibition zone of the prepared foam dressings on various bacteria.

| Formulations | S. aureus (mm) | B. subtilis (mm) | E. coli (mm) | P. aeruginos (mm) |
|--------------|---------------|-----------------|--------------|-----------------|
| Bl-1Ag-AS    | 31.93 ± 3.46  | 25.52 ± 3.35    | 31.57 ± 4.76 | 28.67 ± 2.64    |
| H6-1Ag-AS    | 31.53 ± 2.90  | 27.11 ± 3.29    | 30.23 ± 4.98 | 29.38 ± 2.62    |
| C6-1Ag-AS    | 31.76 ± 4.40  | 29.41 ± 3.33    | 34.71 ± 5.32 | 30.17 ± 4.01    |
| A6-1Ag-AS    | 31.34 ± 3.37  | 27.71 ± 4.84    | 34.02 ± 4.73 | 29.95 ± 4.02    |
| Bl + Ag solution (1 mg/cm²) | 16.13 ± 1.16 | 16.68 ± 1.59 | 18.56 ± 1.24 | 12.24 ± 1.06 |
| Bl           | NZ            | NZ              | NZ           | NZ              |
| H6           | NZ            | NZ              | NZ           | NZ              |
| C6           | NZ            | NZ              | NZ           | NZ              |
| A6           | NZ            | NZ              | NZ           | NZ              |
| Bl-AS        | NZ            | NZ              | NZ           | NZ              |

NZ = no zone of inhibition
the AS which present partial negative charge and lead to prolonging release. In wound environment, the occlusive effect from the dressing involved the entrapment of water result in the rise in temperature and increase hydration of the wound site. This would increase the rate and amount of drug releasing.

3.1.3. Antibacterial test
The results revealed that BI, H6, C6, A6 and BI-AS did not show clear zone inhibition while BI-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS and A6-1Ag-AS exhibited a large clear inhibition zone, which were statistically non-significant (P > 0.05) in every type of tested bacteria (Table 2). The MIC of the silver nanoparticles to P. aeruginosa, S. aureus, E. coli and B. subtilis were in a range of 0.4–3.1 μg/mL [64] while the foam dressing released approximately 4–5 ppm. These clear zones were statistically larger than other tested formulations except the BI foam which was pipetted with Ag solution in 1 mg/cm² (BI + Ag solution), which showed a moderate clear zone area. Large surface area of the silver nanoparticles would have more contact area with the bacteria, thus a higher efficiency in bacteria inhibition. Moreover, the large pore size of the prepared foams facilitated the release of silver. The BI + Ag solution had a smaller clear zone and was significantly different compared to foam dressing impregnated with silver and asiaticoside (P < 0.05). This was possibly due to the silver solution was obstructed within the middle area while pipetting onto the foam sheet.

3.1.4. Cytotoxicity test
The percent cell viability of BI group was slightly less than PBS group (101.16% ± 7.07% and 107.29% ± 9.71%, respectively) (Table 3). No foam adsorb PBS group while other groups contained foam in well plate. The added foam in each well plate might interfere the cell growth. Although TDI in foam formulation was noted to be cytotoxic [65,66], however, TDI could rapidly vaporize under room temperature [67]. In addition, there were reports that the PU foams did not cause any cytotoxicity [68,69]. The natural polyols used in this study were biocompatibility [5,8,70] and presented comparable results implying that these polymers did not cause cytotoxicity. Similar to another study, AS containing wound dressing could increase the viability of the cultured cells [56]. The foam with 5% AS (BI-AS) showed high percent cell viability (113.34% ± 9.97%). The AS concentration was varied from 1–1000 μM. Concentration at 1000 μM could decrease cell viability [19,25]. From the releasing profiles, AS was just in the range of 15–35 μg.

The silver concentrations used in commercial wound dressings were ranged from 0.08–1.50 mg/cm² [71,72] while the silver in the prepared foam formulation was 1.0 mg/cm². From the test, the result of foam with silver and AS could be concluded that combination of both ingredients did not affect cell proliferation. Moreover, the cytotoxicity from in vitro experiment might not confirm the in vivo toxicity. A wound dressing with nanocrystalline silver caused cytotoxicity in cell cultures but the effect could not be observed in mice [73].

From the studies of Part I, the A6-1Ag-AS was chosen for the studies in Part II due to the appropriate adsorption–desorption properties, stability and non-cytotoxicity. It could provide the high silver and asiaticoside releasing profiles. The BI-1Ag-AS was chosen to compare the efficacy of wound healing in the porcine model.

3.2. Efficacy and safety in the animal model (Part II)

3.2.1. Skin irritation test of selected foam dressing on rabbits
There was no redness and swelling on the tested area determined at time 1, 2, 48 and 72 h (Table 4). They were also no dermatologic effects after dressing the application compared to the control side. The total evaluation score was zero in A6-1Ag-AS group and control group. This result was first evidence in which the developed PU foam dressing with Alg and silver nanoparticles plus AS (A6-1Ag-AS) was safe to apply on the skin.

| Table 3 – Percentage of cell viability of various foam dressings. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group           | PBS             | BI              | H6              | C6              | A6              |
| Cell Viability (%) | 107.29 ± 9.71  | 101.16 ± 7.07  | 104.41 ± 8.18  | 102.35 ± 5.42  | 100.01 ± 5.77  |

| Table 4 – The redness and swelling score in the study and control group over 72 h. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group           | 1 h Redness     | 24 h Redness    | 48 h Redness    | 72 h Redness    |
|                 | Swelling        | Swelling        | Swelling        | Swelling        |
| Rabbit 1 – A6-1Ag-AS | 0              | 0              | 0              | 0              |
| Rabbit 1 - Control | 0              | 0              | 0              | 0              |
| Rabbit 2 – A6-1Ag-AS | 0              | 0              | 0              | 0              |
| Rabbit 2 - Control | 0              | 0              | 0              | 0              |
| Rabbit 3 – A6-1Ag-AS | 0              | 0              | 0              | 0              |
| Rabbit 3 - Control | 0              | 0              | 0              | 0              |
3.2.2. **Efficacy and safety of prepared foam sheets on pigs**

The average initial wound areas were $2.02 \pm 0.05, 2.04 \pm 0.17, 2.05 \pm 0.15, 2.03 \pm 0.23$ and $2.02 \pm 0.18$ cm$^2$ in the comparative group I-II, study group I-III which included Bl-1Ag-AS, A6-1Ag-AS and A6-1Ag groups, respectively with no significant difference ($P = 0.995$). The wounds were randomly assigned to be treated with five types of dressings. All wounds were deep partial thickness wounds, which had pathology in the dermis layer.

Fig. 4 shows the appearance of the wounds which received different treatments on 0, 4, 7, 14 and 21 d. These photographs revealed a significant acceleration of the time of wound closure observed in comparative group II and study group II over study group III. At day 7, the granulation tissue was fully grown in study group II which demonstrated the red connective tissue compare to the white and dry wound appearance in study group III. The healthy red color in the new generative tissues over the wound bed could refer to blood supplies that were forming to deliver nutrients to the tissues. The cells in this area formed the extracellular matrix. The white and dry wound bed appearance demonstrated to show the slower rate of wound healing. At day 14 and 21, all groups except study group III show almost completely granulated tissues and epithelialization especially in comparative group II and study group II. It confirmed with the results in Fig. 6; the wound in these two groups were significant healed faster than the wound in study group III. The percentage of the wound closure of comparative groups and study groups are shown in Fig. 5. The average percentage of epithelialization was $84.59 \pm 8.09$ at day 21. Because the deep partial thickness wound was deep, the healing time might take longer than superficial partial thickness wounds. There were no significant differences between the group at day 4, 7 and 14. However, at the 21st d after the creation of the wound, the percentage mean of the

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**Fig. 4 – Wound appearance at day 0, 4, 7, 14 and 21 after treatment of commercial dressings (comparative I and II) compared to foam without natural polyols impregnated with silver and asiaticoside (Bl-1Ag-AS), foam with 6% Alg impregnated with silver and asiaticoside (A6-1Ag-AS) and foam with 6% Alg impregnated with silver (A6-1Ag). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**
Table 5 – The histologic evaluation of the wounds.

| Parameters                      | Histological grading score (Day 7/ Day 14) |
|---------------------------------|--------------------------------------------|
|                                 | Comparative I | Comparative II | Bl-1Ag-AS | A6-1Ag-AS | A6-1Ag |
| Epithelium cell layer           | 1.10 ± 0.99 / | 1.03 ± 0.80 / | 1.48 ± 0.73 / | 1.20 ± 0.90 / | 1.46 ± 0.88 / |
| Amount of inflammatory cell     | 2.28 ± 0.83 / | 2.24 ± 0.85 / | 2.13 ± 0.85 / | 2.65 ± 0.48 / | 2.44 ± 0.67 / |
| Amount of fibroblast            | 1.94 ± 0.74 / | 2.12 ± 0.63 / | 2.00 ± 0.78 / | 2.14 ± 0.67 / | 2.20 ± 0.57 / |
| Amount of new capillary         | 0.91 ± 0.35 / | 0.88 ± 0.40 / | 0.86 ± 0.34 / | 0.88 ± 0.40 / | 0.80 ± 0.51 / |
|                                 | 0.86 ± 0.57 / | 0.82 ± 0.72 / | 0.76 ± 0.69 / | 1.12 ± 0.75 / | 0.92 ± 0.70 / |
|                                 | 1.85 ± 0.55 / | 1.92 ± 0.44 / | 2.10 ± 0.46 / | 2.14 ± 0.57 / | 1.78 ± 0.65 / |
|                                 | 1.28 ± 0.61 / | 1.26 ± 0.60 / | 1.28 ± 0.50 / | 1.38 ± 0.57 / | 1.27 ± 0.57 / |
|                                 | 0.81 ± 0.54 / | 0.80 ± 0.40 / | 0.86 ± 0.42 / | 0.90 ± 0.52 / | 0.82 ± 0.52 / |

* Significance consider, P (0.05, one way ANOVA test, The epithelial cell layer score of Bl-1Ag-AS < A6-1Ag-AS at 14 days, P = 0.01; The amount of fibroblast score of A6-1Ag-AS ) A6-1Ag at 14 d, P = 0.04.

Fig. 5 – Percentage of wound closures of commercial dressings (comparative I and II) compared to PU foam dressings (Bl-1Ag-AS, A6-1Ag-AS and A6-1Ag) for 21 d (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The histological analysis of the wounds, which were evaluated in four parameters: the epithelial cell layer, number of the inflammatory cells, number of fibroblasts and number of capillaries. Comparing the wound lesions between treatment groups, the epithelial cell layers, extracellular matrix and the Masson’s trichrome stained of extracellular matrix were shown in Fig. 6. The normal skin was taken from an area, which had not been wounded including the epithelial cells, collagen bundles, fibroblasts and some of the inflammatory cells and new capillaries as shown on left side of Fig. 6A, 6B and 6C. The three skin layers were epidermis, which is the outer layer, the dermis which is the second thick layer and hypodermis, which mainly consists of adipose tissue. The histologic evaluations on day 7 and 14 including epithelium cell layer, amounts of the inflammatory cells and fibroblasts were reported in Table 5.

The reepithelialization occurred for 7 d (Fig. 6A). There were some epithelial cells from the neighboring epidermis that began to replicate and migrate into the wound bed. In all groups, the average epithelial cell layer notably increased at day 14 compared to day 7 (P < 0.05). This might have been caused by the epithelial cell growth covering the wound bed, which would prevent dehydration and protect the wound externally. At day 14, the study group I had an epithelial cell layer score significantly less than study group II (P < 0.05). The alginate in study group II might be a reason to keep hydration and facilitate the proliferation of the epithelial cell. In addition, AS might be involved in this process. Cheng et al. [18] reported that this compound could activate intestinal epithelium cell growth.

Lots of inflammatory cells were observed on day 7 and dramatically decreased in day 14 (P < 0.05) (Fig. 6B). The inflammatory phase normally occurred within the first week after injury. Macrophage and neutrophil chemotaxis would remove debris cells and bacteria. After that, the extracellular matrix was produced in the proliferative phase in which the inflammatory cells would have less importance. Although there was some evidence about the Centella asiatica extract reducing inflammation [75,76], there was no significant difference in the inflammatory cells score between the groups at each point of time (P > 0.05). This might be because these wounds were deep partial thickness wounds, and the inflammation might be greater than a superficial partial thickness wound.

wound’s closure was significantly faster in the study group II and comparative group II than in study group III (P = 0.04 in both pairs). Study group I showed a smaller closure in size than study group III, but there was no statistical significance. It might have been caused by the AS in Bl-1Ag-AS and A6-1Ag-AS groups that could promote the healing process. Lee et al. [19] reported that this compound could stimulate the migration of epithelial cells. Moreover, the comparative groups I and II had a smaller pore size than the study groups. This might retain more hydration than the study groups. The moist wound could heal faster than a dry wound because the epithelial cells could migrate easily [74]. Moreover, comparative group II had an alginate content, which was the hydrophilic polymer; this might facilitate moisture at the wound bed and could detect the difference between comparative group II and study group III.

The positive effects of AS on wound healing, especially in reepithelialization and reparation were found in the
Moreover, itching of the wound might occur during the healing process, so the animal might scratch and let inflammatory cells be released in all the wounds.

At day 7, all the wounds had some granulation tissue. The collagen which was exhibited in pink fiber mixed with the fibroblasts showed as purple satellite-shaped cells. The amount of the fibroblasts increased in day 14 compared to day 7, especially in comparative group II and study group II ($P = 0.02$ in both pairs). At day 14, there were more fibroblasts found in study group II than those in study group III ($P < 0.05$). The collagen fiber became denser, which was the signs of regeneration of the dermis. This result confirmed the findings of previous studies [21,77] in which AS activates fibroblast proliferation. The increasing of the fibroblasts led to an increase of the collagen fibers and wound’s strength. The collagen fiber should be confirmed by the photos from the histologic stained with Masson’s trichrome in which the collagen is stained in blue (Fig. 6C). The wound tissue from study group III had loose collagen fiber in both points of time when compared to other groups.

The new capillaries were found in day 14 less than day 7 (Fig. 6B). As a result of the nearly completed healing, and the nutrients and oxygen were a lesser necessity. Even though some data showed the AS activated angiogenesis [18,78], there was no difference in these study groups. Also, other factors might be involved in the wound healing; such as animal genetics, food and water consumption, and self-traumatized site from the animal.

There was a dermatologic effect in comparative group I. There were some rashes on the skin surface around the wound. This may have been caused by the adhesive layer that recovered after discontinuing the dressing. Therefore, there was no dermatologic effect found in the study groups.
This study was performed in deep surgical wounds which were clean wounds. However, they could be infected due to animal behavior. The pigs usually scratched the wounds on the wall and the floor. The microbe might infect easily. The silver in PU dressing prevented the infection which might occur. Without infection, the wound could heal continuously without dermatologic reaction. Combination of silver and AS in PU foam would present satisfied results. The percentages of the wound’s closure and histological data supported that the PU foam dressing with alginate and silver plus AS (A6-1Ag-AS) could accelerate wound healing through the migration of the epithelial cells and the proliferation of the fibroblast in a deep partial thickness wound of a porcine model.

4. Conclusion

The PU foam sheets could be successfully prepared with the addition of natural polyols. The investigated natural polyols especially alginate and hydroxypropyl methylcellulose improved the foam characteristics with the increase in the absorption property and compressive strength. All formulations confirmed comparable antimicrobial effect in the disk diffusion test and showed non-cytotoxicity. The foam dressing with A6-1.0Ag of silver showed the highest silver releasing results and subsequently presented satisfactory releasing of AS. This formulation (A6-1Ag-AS) could improve wound healing in both the percentages of wound closure and histological parameters in porcine model with deep partial thickness wound. The wound applied with A6-1Ag-AS presented the epithelial cells and fibroblasts notably proliferated and repaired the lesion. This also demonstrated a gentle skin effect on a rabbit model. Formulation of A6-1Ag-AS could be an alternative dressing for wound treatment. The efficacy and safety in clinical studies was planned to further investigated.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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