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DEVELOPMENT AND PHYSICAL CHARACTERIZATION OF TOPICAL WOUND DRESSING FORMULATIONS FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITES

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ABSTRACT

Skin transplant patients require wound treatment for both graft and donor sites, including managing pain, preventing infection, and ensuring proper healing. This project aims to develop a topical wound dressing formulation for skin graft donor sites in the form of a hydrocolloid film to solve the problems related to the leakage of drug solutions during patient treatment. The hydrocolloid film used low methoxyl pectin (LMP), sodium carboxymethyl cellulose (SCMC), and gelatin in a specific ratio using the casting technique. The formulation was compared with and without glutaraldehyde (Glu) as a cross-linking agent and a drug cocktail that includes bupivacaine, adrenaline, and tranexamic acid, which has bleeding control, and local anesthetic properties, was added. The chemical and physical properties of the developed hydrocolloid films were evaluated across various parameters. The results showed that all hydrocolloid films have flexibility and translucency, with thickness ranging from 0.28±0.05 to 0.38±0.05 mm. The pH values were in the appropriate range for the skin. The fluid uptake capacity ranged from 42.56±8.72 to 64.79±24.44%, and the water vapor transmission rates were between 2112±71.17 and 2284±204.13 g/m^2/day. Incorporating glutaraldehyde resulted in a darker yellow color of the film and required greater force to rupture compared to formulations lacking glutaraldehyde. Furthermore, infusing the drug into the hydrocolloid film lowered the pH values and the film's integrity and led to a shorter dissolution time, possibly due to increased moisture absorption. In summary, the hydrocolloid film formulations with and without glutaraldehyde exhibit suitable physical properties as topical wound dressings for skin grafts. The choice of formulation can depend on the desired duration of use. However, further safety testing on patients is recommended before clinical application.

Keywords: topical wound dressing, hydrocolloid films, skin grafts, casting technique, low methoxyl pectin, sodium carboxymethyl cellulose, gelatin

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Introduction

Skin grafting is used to treat burn wounds, particularly full-thickness deep burns,[1] which are injuries that penetrate deeply enough to destroy the epidermis and dermis, sweat glands, and nerve cells. Patients often feel no pain in these wounds and may have burns extending down to the muscle or bone. The scars appear white, pale, yellow, brown, or black, thick and hard like leather. This type of wound cannot heal independently, making skin grafting essential. Consultations with plastic or general surgeons can be treated using a split-thickness skin graft (STSG) technique, where the skin is harvested from a donor site for coverage of the wounds. This harvesting process creates a wound in the donor area, accompanied by bleeding, pain, and serum leakage. Typically, surgeons use an adrenaline-diluted solution with normal saline (NSS) soaked gauze to cover the harvested skin area to prevent further bleeding. Due to the pain caused by skin harvesting, bupivacaine, an anesthetic, is also topically administered for pain relief. However, its duration of action is only 4 to 6 hours, often necessitating the reopening of the patient's wound to topically administer bupivacaine again, potentially increasing their pain from the frequent reopening.

Additionally, since this mixed medication is in a clear solution form, it may leak during topical administration, resulting in suboptimal efficacy, necessitating frequent doses, and causing increased irritation and pain for the patient. The identified issues include drug leakage and the requirement for frequent topical administrations, contributing to the patient's pain and wound irritation.

Wound healing is a complex process that requires an appropriate environment to facilitate proper and rapid healing. Wound healing occurs in 4 stages: (1) the hemostasis phase, which happens immediately after the injury; (2) the inflammation phase, which occurs shortly after tissue injury and can cause swelling in the wound area (3) the proliferation phase; where new tissue and blood vessels begin to form, and (4) the remodeling phase, where the tissue is fully repaired. Dressing a wound is a form of care that can help promote faster healing by being able to maintain moisture.[1] Various types of wound dressings are currently available. Therefore, selecting the appropriate dressing for the kind of wound is essential for optimizing wound treatment.

Hydrocolloid dressings are popular because they typically contain polymers such as carboxymethyl cellulose, gelatin, and pectin. They resemble non-permeable gel sheets while allowing vapor to escape. When applied to a wound, hydrocolloid dressings form a gel that maintains moisture, absorbs exudate, removes necrotic tissue, and is painless when removed. They are often used for wounds with low to moderate exudate, minor burns, and necrotic tissue wounds requiring moisture for autolysis. This is better than traditional wound dressings, such as gauze, which dry out and adhere to the wound. Hydrocolloid dressings with acidic pH levels are recommended to minimize infection, transforming into a gel upon contact with the wound, which prevents bacteria and retains moisture.[2]

The medication used for skin graft site wounds includes a drug cocktail containing bupivacaine.[3-5] It is soluble in water and alcohol and slightly in chloroform and acetone. It remains stable in acidic conditions within a pH range of 4.5 to 6.0. The injectable form of the drug should be stored at a temperature between 20 and 25°C. It is compatible with normal saline and dextrose solutions. The drug is resistant to temperatures up to 40°C for short periods; however, exposure to heat and light should be minimized. Bupivacaine serves as a local anesthetic for pain relief in surgical areas. Adrenaline or Epinephrine is very slightly soluble in water and in alcohol, insoluble in ether, in chloroform, and fixed and volatile oils. It is sensitive to light and oxidation. It is recommended to store it away from light and oxygen, which causes it to turn pink and then brown. Do not use the solution if it changes color or precipitates. Adrenaline is used for treating

anaphylaxis and severe allergic reactions.[4,6,7] Tranexamic acid is freely soluble in glacial acetic acid, practically insoluble in acetone, and 96% ethanol. Tranexamic acid is a drug that prevents thrombolysis, which is used to treat bleeding.[8,9]

This study aims to develop a localized wound dressing material for skin graft areas as a hydrocolloid film compatible with human skin, addressing the challenges of drug leakage during topical administration. It is anticipated that when developed into a hydrocolloid film formula, the active ingredient will have a longer duration of action, thereby reducing the frequency of mixed drug administration, which in turn minimizes the need for reopening and closing the wounds, ultimately resulting in less suffering and irritation for the patient. Furthermore, creating localized wound dressing materials in hydrocolloid film can help retain moisture at the wound site and absorb exudate from the wound.

Materials and Methods

1. Materials

Low methoxyl pectin: LMP UnipectineTM OF 300 C was purchased from Cargill™ (Saint Germain, France), Sodium carboxymethyl cellulose: SCMC was purchased from Union science (Chiangmai, Thailand), Gelatin (Limed bone gelatin BP 250 bloom) was purchased from Nitta Gelatin Inc. (Osaka, Japan), Glycerin (Refined Glycerine) was purchased from Srichand United Dispensary Co., Ltd. (Bangkok, Thailand), Glutaraldehyde solution 25% was purchased from Appichem Panreac ITW Companies, Bupivacaine (Bupivacaine Injection BP 0.5%) was purchased from aspen AstraZeneca AB (Sodertalje, Sweden). Deionized water served as the solvent for preparing hydrocolloid film.

2. Preparation of hydrocolloid film

LMP, gelatin, and SCMC were weighed (1 g each), then mixed roughly, and the mixture was sprinkled into a beaker containing 50 ml of distilled water and slowly sprinkled the mix while being stirred at 750 rpm using a magnetic stirrer (ECOplatePlus, TOPSCIEN, Ningbo, China) at 55°C. Be careful to prevent clumping. The sprinkling process took approximately 10 minutes. Once the substances were well dispersed, 10 g of glycerin was added, the beaker was covered with foil, and stirring was continued for 2 hours at 750 rpm and 55°C to obtain a clear yellowish solution with some small bubbles. Then, the solution was stirred gently at approximately 50 rpm without heat for 10 minutes to reduce the temperature to room temperature. Next, a 0.15 g solution of 25% glutaraldehyde was prepared in about 5 ml of distilled water and incorporated into the cooled solution, and stirred the mixture at 500 rpm until it was well combined. Then, the drug cocktail containing bupivacaine, adrenaline, and tranexamic acid was added to the mixture and stirred to combine, and the weight was adjusted to 100 g with distilled water. Stirring was continued at 750 rpm for 15 minutes without heat. Afterward, the solution was transferred into 50-ml centrifuge tubes, 20 g per tube, filling four tubes. The foam was removed using a diaphragm vacuum pump (GM-0.5A model, Linhai Tanshi Vacuum Equipment Co., Ltd., Linhai, China) for 10 minutes. If foam remained, vacuuming was continued for another 10 minutes. The solution was poured onto plastic trays (6 × 9.5 cm), 20 g per tray. The trays were dried at 40°C for 48 hours (Figure 1).

3. Hydrocolloid film characterizations

3.1 Viscosity and the flow pattern of polymer solutions

The flow and viscosity of the polymer solution sample 0.5 ml before drying were evaluated using a Brookfield Rheometer (Model R/S, Brookfield Engineering, Massachusetts, USA) equipped with a 25 mm diameter parallel plate at an ambient temperature of 25 ± 1°C to be used for quality control and to observe the relationship with other parameters. The test protocol involved a step time of 60 seconds and included measurements taken at 30 points, resulting in a final value of 600 D[1/s]. The Rheo 2000 Version V2.8 program facilitated the analysis.

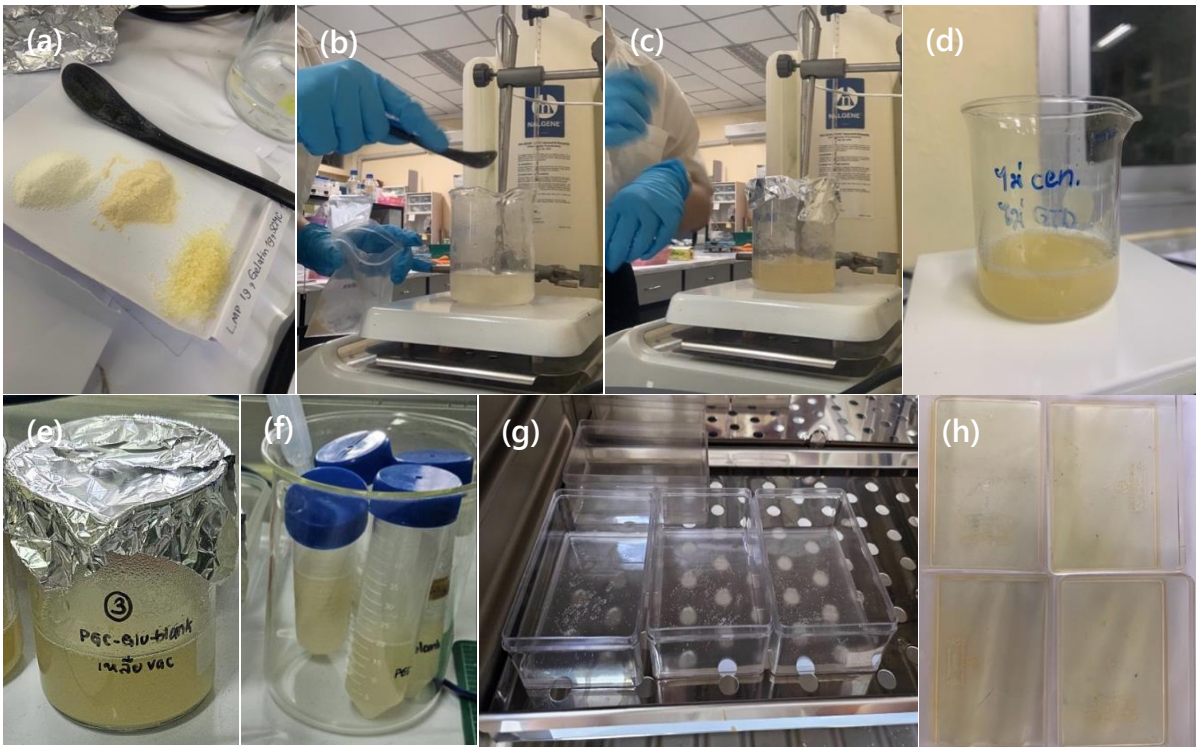


Figure 1 The method for preparing hydrocolloid film was as follows: weighed the ingredients **(a)**, the ingredients was sprinkled into the water **(b)**, glycerin was added and stirred for 2 hours **(c)**, stirring was continued at low speed to reduce the temperature **(d)**, glutaraldehyde and the drug was added, and adjusted the volume with water **(e)**, the foam was removed using a vacuum pump **(f)**, the solution was poured into plastic molds and was dried at 40°C for 48 hours **(g)**, Completed hydrocolloid film **(h)**.

Measurements were conducted in triplicate, and the average values for viscosity and flow were calculated.

3.2 Morphological characterization of hydrocolloid films

The morphological characteristics of the hydrocolloid film were evaluated by cutting it into pieces measuring 0.5 cm by 0.5 cm. The film was then analyzed using a Scanning Electron Microscope (SEM) JEOL NeoScope™ Benchtop (Tokyo, Japan). The cross-section and surface morphology of the hydrocolloid film were examined, with images obtained at a magnification of 150×; the accelerating voltage at 15 kV was used for SEM imaging.

3.3 Thickness of the hydrocolloid film

The average thickness of the hydrocolloid film was measured using a Mitutoyo Micrometer (Model 293-240-30, range 0-25 mm, Mitutoyo, Tokyo, Japan). Random measurements were taken 5 times on each sheet. The five values obtained were averaged, and the standard deviation was calculated. The measurements were conducted in triplicate.

3.4 Mechanical strength test

Evaluate the texture of the hydrocolloid film by testing the pressure applied and calculating the tensile strength, elongation at break, and Young's modulus for each film formulation using a Texture Analyzer (TA) from Stable Micro Systems (Godalming, England). This involved cutting the hydrocolloid film into 2 × 2 cm^2 pieces, with three samples per formulation (three repetitions). The parameters were set as follows: compression mode, flatted probe p/2 (diameter 2 mm), the probe's test speed when contacting the sample surface was 2.5 mm per second, and the depth to which the probe pressed into the sample from the contact point was 10.0 mm. Puncture strength, elongation at break, and Young's modulus were evaluated.

3.5 Measurement of pH

Measure the sample's pH using the Compact pH meter model LAQUAtwin-pH-22, Brand HORIBA (Kyoto, Japan). A 0.1 g sample was dissolved in 5 ml of water to ensure that free hydrogen ions (H^+) can be detected, measured 3 times, and then calculated the average.

3.6 Determination of water vapor transmission rate (WVTR)

The water vapor transmission rate (WVTR) was investigated by attaching hydrocolloid film to the mouth of a cylindrical glass bottle with a 17 mm diameter containing 10 ml of water. The mouth of the bottle was sealed with parafilm to prevent moisture loss, and the bottle was stored in an incubator at 37 °C with constant relative humidity. The water evaporation through the hydrocolloid film was measured by weighing the bottle over 7 days. The weight change will indicate water loss. Plot a weight loss (g) graph against time (h) to obtain the slope value. The WVTR can be calculated using equation (1).[10]

$$\text{WVTR } (g/m^2/day) = \frac{(\text{slope} \times 24)}{A} \quad (1)$$

Where A is the area of the sample (m^2), repeat this process 3 times.

3.7 Determination of fluid uptake capacity

The fluid uptake capacity of hydrocolloid films was analyzed using the gravimetric method by cutting the hydrocolloid films into pieces measuring 2 × 2 cm^2; then, the dry weights were measured. Next, the samples were immersed in 15 ml of phosphate buffer saline (PBS) at pH 7.4 for 1 minute at room temperature. Once the swelling was complete, the hydrocolloid film was removed from PBS, gently blotted with filter paper to eliminate excess PBS, and the wet weights were measured. The samples were immersed again, and the weight of the hydrocolloid films was recorded until it reached swelling equilibrium (the weight of the hydrocolloid film did not increase) or the film was damaged. Repeat this procedure three times. The equilibrium of fluid is calculated using the following equation (2).

$$\text{Equilibrium of fluid } (\%) = \frac{\text{Wet weight - Dry weight}}{\text{Dry weight}} \times 100 \quad (2)$$

3.8 Determination of integrity value

The integrity value of the hydrocolloid film was evaluated by cutting samples measuring 2 × 2 cm^2 and recording the initial weight (Wi). Next, the sample was placed into a glass bottle containing 30 ml of PBS and mixed with a low-speed shaker for 1 hour. Afterward, the sample was removed and filtered using vacuum filtration and dried in an oven at 65 °C for 24 hours. Then, the dried sample was weighed (Wd), and the integrity value was calculated using equation (3). Repeat the test 3 times to obtain the average.

$$\text{Integrity } (\%) = \frac{w_d}{w_i} \times 100 \quad (3)$$

3.9 Moisture uptake

Investigate the moisture uptake property of hydrocolloid films by the samples measuring 2 × 2 cm^2 that were cut and dried in an oven at 105 °C for 3 hours. The dry weight (W_1) was measured, and the samples were stored in a closed cabinet at a constant temperature of 25 ± 1°C and constant humidity (60% RH) for 6 days. The hydrocolloid film on days 2 and 6 (W_2) was weighed. The moisture uptake value was calculated using the following equation (4). Repeat the process 3 times and determine the average.

$$\text{Moisture uptake } (\%) = \frac{W_2 - W_1}{W_1} \times 100 \quad (4)$$

4. *In vitro* dissolution of hydrocolloid film

To test the *in vitro* dissolution of the hydrocolloid films, each film was cut into 2 × 2 cm^2. Then, the film was soaked in a 7 ml PBS solution at room temperature, ensuring the solution covered the hydrocolloid film. The dissolution patterns of each formulation were visually recorded, and took pictures of the hydrocolloid film at 0, 0.1, 1, 2, 16, 20, and 24 h, and dissolution patterns of each hydrocolloid film were compared. It is considered completely dissolved if it dissolves into a clear solution without any hydrocolloid film residue. The experiment was conducted in triplicate.

5. Drug content

Determine the drug content by weighing the hydrocolloid film with the active ingredient, and it was dissolved in a 0.9% normal saline solution to achieve a bupivacaine concentration of 500 mg/ml. When the hydrocolloid film was completely dissolved, the polymer was centrifuged at 5000 g for 25 minutes using the MPW-352 centrifuge, MPW MED Instrument (Warszawa, Poland). The clear upper solution was filtered through a 0.45-µm filter, and then the substance's absorbance was measured using a UV-Vis spectrophotometer (UV2600i Shimadzu Kyoto, Japan) at a wavelength of 263 nm. The blank formulations were dissolved in a saline solution to be used as a blank solution, and the obtained absorbance values were compared with the concentration calibration curve.

6. Cell cytotoxicity

The MTT assay used human keratinocyte cells (HaCaT cells) to test cell cytotoxicity. HaCaT cells were seeded in 96-well plates at a density of 8×10^3 cells per well and incubated for 24 h in Dulbecco's Modified Eagle's Media. The sample was filtered to sterilize through a 0.22 µm membrane filter and incubated with cells for 24 h at 37°C, 5% CO_2. After incubation, samples were removed. MTT at a concentration of 0.5 mg/mL was added (100 µL/well) and incubated at 37 °C for 2 h. Then, the medium was replaced with DMSO (100 µL) to solubilize the formazan product. The absorbance for each well was measured at 550 nm using a mi-croplate reader (Spectramax M3, Molecular Devices, San Jose, USA). Afterward, cell toxicity was assessed, triplicates, and cell toxicity and cell viability were calculated using the equation (5) and (6), respectively.

$$\text{Cell cytotoxicity (\%)} = \frac{A - B}{A} \times 100 \qquad (5)$$

$$\text{Cell viability (\%)} = 100 - \text{cell cytotoxicity} \qquad (6)$$

Where, A and B were the absorbances of the control and test wells.

7. Statistical analysis

The study results were presented as mean ± standard deviation. A one-way ANOVA was used for evaluation. Tukey's test was used to evaluate differences among the means, with statistical significance set at a 95% confidence level (p-value < 0.05). Statistical analysis was conducted using Jamovi version 2.3.19.0 (Jamovi Inc., Sydney, Australia).

Results and Discussion

1. Viscosity and the flow pattern of polymer solutions

The average (Eta all step) and standard deviation of viscosity for the PGC-blank, PGC-Glu-blank, PGC-D, and PGC-Glu-D formulations were 0.384 ± 0.021, 0.406 ± 0.008, 0.328 ± 0.016, and 0.351 ± 0.018 [Pa•s], respectively, as shown in Table 1. The formulations that included the drug cocktail, PGC-D and PGC-Glu-D, showed significantly lower average viscosity compared to the formulation without the drug cocktail (p = 0.002 and 0.015, respectively). The graphs of Eta [Pa•s] versus D [1/s] (viscosity versus shear rate) and Tau [Pa] versus D [1/s] (shear stress versus shear rate) can indicate the rheological behavior of the solutions, in Figure 2, all samples demonstrated non-Newtonian pseudoplastic flow behavior. The results indicate that when comparing formulations with and without glutaraldehyde, the formulation containing glutaraldehyde acts as a cross-linking agent, resulting in higher viscosity, though not significantly so. However, the presence of the drug cocktail in the formulation significantly reduced viscosity, possibly because the drug interferes with cross-linking or lowers the formulation's pH, leading to decreased viscosity.[11] The impact of flow and viscosity is beneficial for solution quality control on film quality in terms of film thickness, which will affect other film properties such as mechanical property, fluid uptake capacity, and integrity.

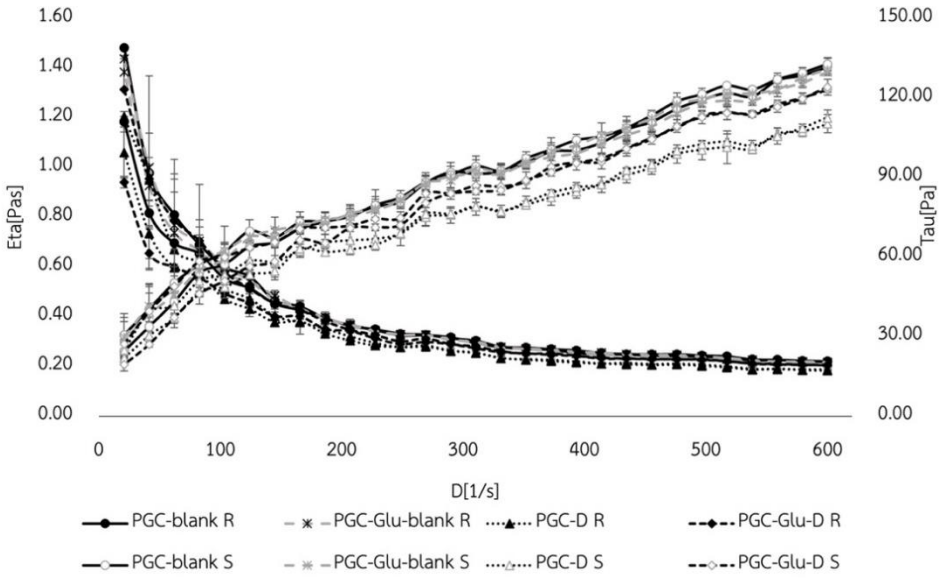


Figure 2 A graph between Eta[Pa•s] and D[1/s] (PGC-blank R, PGC-Glu-blank R, PGC-D R and PGC-Glu-D R) and a graph between Tau[Pa] and D[1/s] (PGC-blank S, PGC-Glu-blank S, PGC-D S and PGC-Glu-D S).

Table 1 The viscosity of polymer solution.

Sample	Composition	The average viscosity)Eta all step) [Pa×s]
PGC-blank	LMP, gelatin, and SCMC	0.384 ± 0.021[a]
PGC-Glu-blank	LMP, gelatin, SCMC and Glu	0.406 ± 0.008[a]
PGC-D	LMP, gelatin, SCMC and drug cocktail	0.328 ± 0.016[b]
PGC-Glu-D	LMP, gelatin, SCMC, Glu and drug cocktail	0.351 ± 0.018[b]

Average values with a different letter ("a", "b", "c") show significant difference between each sample. In contrast, average values with the same letter are not statistically different (p < 0.05) according to Tukey's test.

2 Properties of hydrocolloid film

2.1 Morphological characteristics of hydrocolloid films

The preparation of hydrocolloid films using the casting method resulted in smooth, flexible, and bubble-free films. As seen in Figure 3 (left and middle), the external appearance of the hydrocolloid films from each formulation appears similar to the naked eye. However, the PGC-Glu-blank exhibits a darker color than the PGC-blank, and the PGC-Glu-D shows a darker color than the PGC-D. This indicates that formulations containing glutaraldehyde as a cross-linking agent have a darker hue than those without it. The color variation is believed to be due to glutaraldehyde in the formulation, as it reacts with protein to yield a yellow color. An analysis[12] of the morphological characteristics of the hydrocolloid films using a Scanning Electron Microscope (SEM) at 150x magnification demonstrated that all formulations featured a dense texture without pores, as illustrated in the cross-sectional images of the hydrocolloid films in Figure 3 (right).

2.2 Thickness of the hydrocolloid film

The thickness of the hydrocolloid films in the four samples, illustrated in Figure 4, indicated that the thickness of PGC-Glu-D was significantly greater than PGC-blank. The thickness measurements were as follows: PGC-blank was 0.28 ± 0.05 mm, PGC-Glu-blank was 0.31 ± 0.04 mm, PGC-D was 0.31 ± 0.06 mm, and PGC-Glu-D was 0.38 ± 0.05 mm. This

suggests that the addition of the drug cocktail, which includes bupivacaine or glutaraldehyde, did not influence the thickness of the hydrocolloid film. However, it was observed that the thickness of the hydrocolloid film may not be consistent across the same sheet.. This inconsistency may arise from the casting method employed; placing it on an uneven surface during drying can lead to variations in the hydrocolloid film's thickness. This issue could be remedied by utilizing more precise molding tools.

2.3 Mechanical properties and pH value of hydrocolloid film

The results of the texture analyzer test, including the puncture strength, elongation at break, and Young's modulus of each hydrocolloid film formulation are shown in Table 2. If the tensile strength,

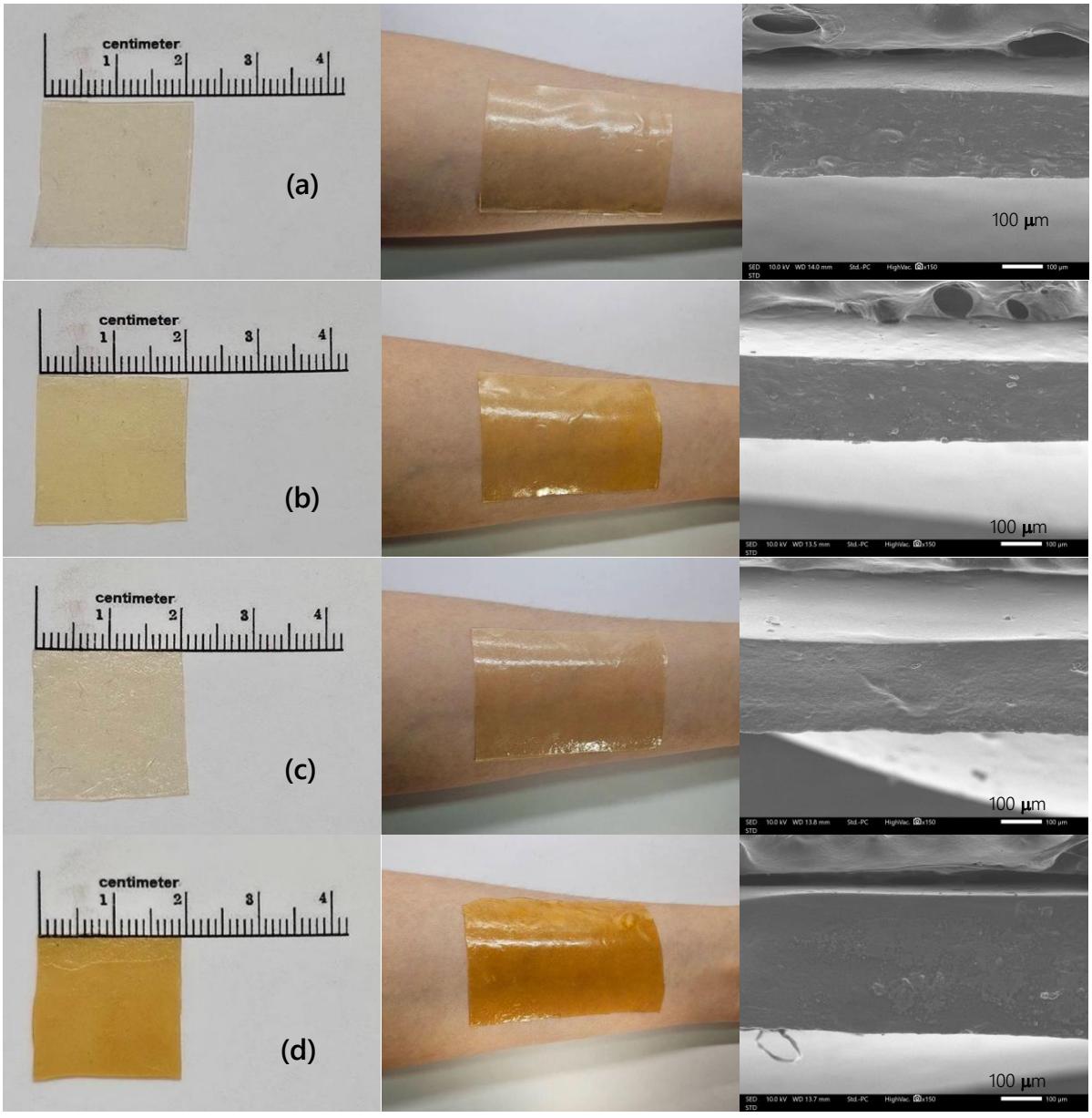


Figure 3 The external characteristics of the hydrocolloid film (left and middle) and the cross-sectional view of the hydrocolloid film from a scanning electron microscope at 150x magnification (right) of the PGC-blank **(a)**, PGC-Glu-blank **(b)**, PGC-D **(c)**, and PGC-Glu-D **(d).**

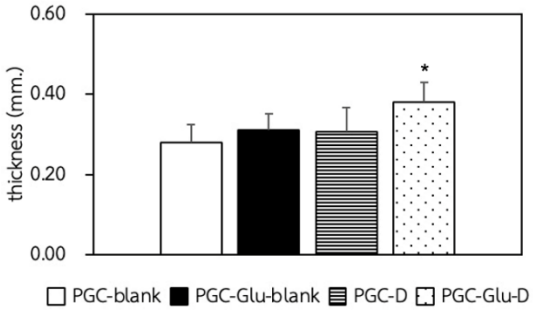


Figure 4 Thickness of hydrocolloid films (*refer to statistically significant as $p < 0.05$).

Table 2 Puncture strength, percentage of elongation at break, Young's modulus and pH of hydrocolloid film.

Sample	Thickness (mm)	Mean ± SD			
		Puncture strength (N/mm^2)	% Elongation at break	Young's modulus (N/mm^2)	pH
PGC-blank	0.28 ± 0.05[a]	0.16 ± 0.02[a]	31.35 ± 4.70[a]	235.79 ± 16.38[a]	4.97 ± 0.04[a]
PGC-Glu-blank	0.31 ± 0.04[ab]	0.21 ± 0.02[b]	53.22 ± 6.85[b]	223.34 ± 22.76[a]	4.94 ± 0.05[a]
PGC-D	0.31 ± 0.06[ab]	0.15 ± 0.02[a]	37.38 ± 4.92[a]	202.67 ± 18.86[b]	4.82 ± 0.00[b]
PGC-Glu-D	0.38 ± 0.05[b]	0.25 ± 0.02[c]	42.66 ± 3.28[c]	300.72 ± 38.61[c]	4.80 ± 0.02[b]

Average values with a different letter ("a", "b", "c") show significant difference between each sample. In contrast, average values with the same letter are not statistically different ($p < 0.05$) according to Tukey's test.

elongation at break, and puncture resistance values are high, the hydrocolloid film will be difficult to tear or crack during use. The formulations containing glutaraldehyde as a cross-linking agent, PGC-Glu-blank and PGC-Glu-D, exhibit higher puncture strength, elongation at break, and Young's modulus values than the formulations without glutaraldehyde. This is attributed to the presence of glutaraldehyde in the formulations, which enhances the viscosity and resistance to compression of the hydrocolloid films, resulting in higher values than those without glutaraldehyde.[13] This occurs because glutaraldehyde can cross-link with gelatin[14] as illustrated in Figure 5.

The pH results showed that the formulations with drugs, such as PGC-Glu-D, had significantly lower pH than PGC-blank (4.80 ± 0.02 versus 4.97 ± 0.04, $p = 0.025$). PGC-Glu-blank was 4.94 ± 0.05, and PGC-D was 4.82 ± 0.00, as shown in Table 2. The skin's pH is approximately 4-6.[15] Therefore, the hydrocolloid film has an appropriate pH for use on the skin.

2.4 WVTR

The WVTR of PGC-blank, PGC-Glu-blank, PGC-D, and PGC-Glu-D showed no significant differences ($p = 0.550$), with values of 2261.09 ± 92.33, 2284.26 ± 204.13, 2226.74 ± 193.46, and 2112.22 ± 71.17 g/m^2/day, respectively (Figure 6). The lack of difference in WVTR among the four samples may be due to the similar morphological characteristics of the hydrocolloid films, which have comparable porosity,[17] as observed from the results of SEM. Additionally, the water vapor evaporates at a constant rate, as indicated by the graph of the remaining weight (Figure 7). A high WVTR will promote faster wound healing but may also leave a scar. Conversely, a low WVTR will slow wound healing

and increase the risk of bacterial infection.[18] Generally, the appropriate WVTR depends on the depth of the wound; the deeper the wound, the higher the WVTR should be.[19] According to the study by Queen et al., the WVTR should fall within the range of 2,000–2,500 g/m²/day.[20]

2.5 Fluid uptake capacity of hydrocolloid film

In this study, the fluid uptake capacity of hydrocolloid films was tested by immersing the hydrocolloid films in a PBS solution at room temperature for 1 min, as the films became damaged or incomplete after 2 min of testing. The results showed that the fluid uptake capacities of PGC-blank, PGC-Glu-blank, PGC-D, and PGC-Glu-D were not significantly different (p = 0.456), with values of 64.79±24.44, 56.84±17.29, 42.56±8.72, and 42.74±21.86%, respectively (Figure 8). This may be due to the similar density and porosity of the hydrocolloid films.[21]

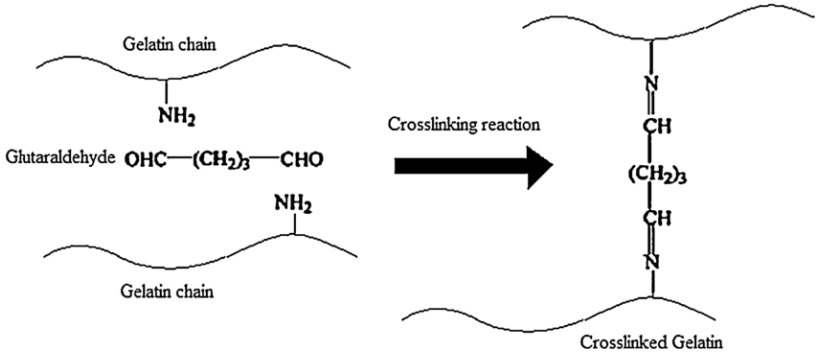


Figure 5 Crosslinking between glutaraldehyde and gelatin.[16]

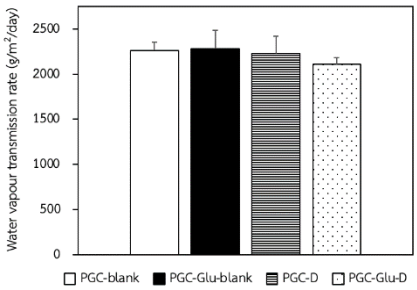


Figure 6 WVTR of hydrocolloid film.

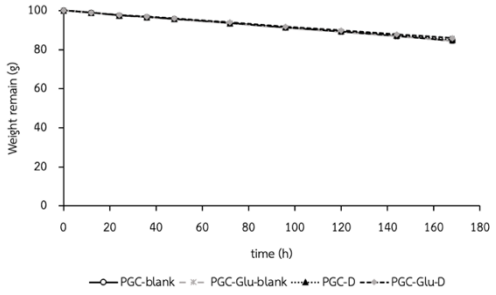


Figure 7 Graph of remaining weight (g) versus time (h).

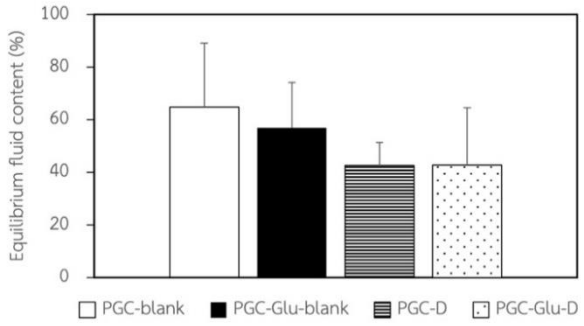


Figure 8 Fluid uptake capacity of hydrocolloid film.

2.6 Integrity value of hydrocolloid film

It was found that formulations with an added drug cocktail had significantly lower integrity values than those without added drugs. The PGC-blank and PGC-Glu-blank showed no significant difference in integrity values (25.45 ± 3.33 and 20.30 ± 0.66, respectively, p = 0.27). However, PGC-D and PGC-Glu-D had significantly lower integrity values compared to the PGC-blank and PGC-Glu-blank, respectively (5.94 ± 0.27 and 7.85 ± 0.39, p = 0.023 and p < 0.001, respectively) as shown in Figure 9, which may be due to the drug cocktail interfering with the film cross-linking.[11] The integrity of the hydrocolloid film is essential, depending on its application. According to a study by Olatunji Ajiteru, the integrity value of commercially available films is approximately 89.65 ± 0.22%.[22] However, since this study requires the hydrocolloid film to dissolve within 24 hours, having a lower film integrity value is advantageous.

2.7 Moisture uptake property

From the experiment, it was found that on day 2, the moisture uptake of PGC-blank, PGC-Glu-blank, PGC-D, and PGC-Glu-D were not significantly different (p = 0.953), with values of 29.43 ± 1.42, 30.67 ± 5.56, 29.96 ± 0.52, and 29.88 ± 1.94, respectively. On day 6, only PGC-D had a significantly higher moisture uptake than PGC-Glu-blank (36.82 ± 0.37 and 34.29 ± 0.44, respectively, p = 0.006). This may be due to the formulation's ability to form bonds with water, enhancing its solubility, as bupivacaine can form hydrogen bonds.[23]

3. *In vitro* dissolution of hydrocolloid films

The *in vitro* dissolution of each hydrocolloid film when submerged in a liquid medium (Table 3) show that the formulations that dissolved best in PBS solution were PGC-Glu-D and PGC-D, which completely dissolved within 20 h. However, at 24 h, the PGC-blank and PGC-Glu-blank had not completely dissolved. The reason that the PGC-Glu-D and PGC-D dissolved faster than the PGC-blank and PGC-Glu-blank may be due to the drugs in the formulations interfering with the cross-linking reaction between glutaraldehyde and the polymer.[18,24,25] Additionally, PGC-Glu-blank dissolves more slowly than PGC-blank, which may be due to glutaraldehyde acting as a cross-linking agent in the formulation, resulting in a more cohesive and resistant hydrocolloid film that dissolves more slowly.[26]

4. Drug content in the hydrocolloid films

From the analysis of drug content, as shown in Table 4, it was found that the PGC-D had a % recovery of bupivacaine 102.36 ± 0.03, while the PGC-Glu-D had a % recovery percentage of bupivacaine 104.49 ± 0.36. It shows that the formulation still contains the active ingredient, and the % recovery being greater than 100% may be due to the blank formulations dissolving harder than the formulations with the drug cocktail.

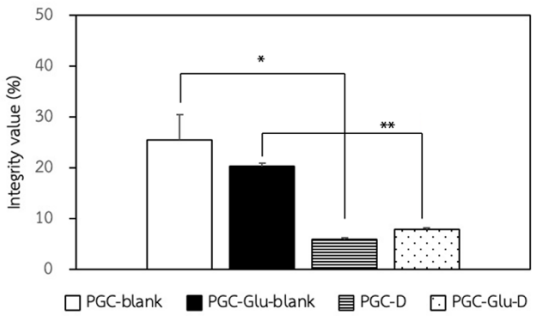


Figure 9 Integrity of hydrocolloid film (* Shown significant difference in integrity values between PGC-blank and PGC-D, ** shown significant difference in integrity values between PGC-Glu-blank and PGC-Glu-D).

Table 3 The *in vitro* dissolution of hydrocolloid films.

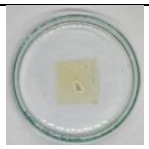
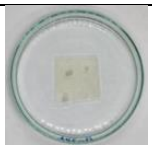
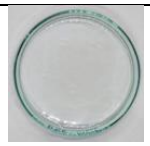
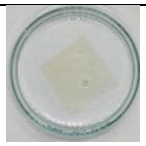
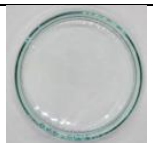
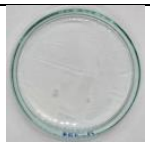
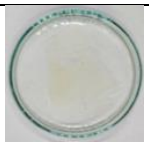
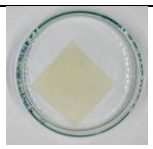
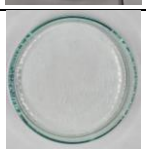
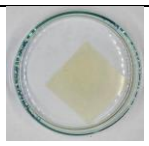
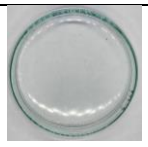
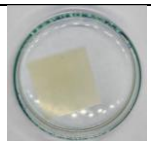
Formulation Time (h)	PGC-blank	PGC-Glu-blank	PGC-D	PGC-Glu-D
0				
0.1				
1				
2				
16				
20				
24				

Table 4 The amount of drug in the hydrocolloid film.

Sample	Concentration of Bupivacaine (mg/ml)	%Drug content
PGC-D	511.79 ± 0.16	102.36 ± 0.03
PGC-Glu-D	523. 36 ± 1.82	104.49 ± 0.36

5. Cytotoxicity

The cell cytotoxicity tests found that formulations with added drugs had lower cell viability than those without added drugs, as shown in Table 5. This occurs because chemicals reduce cell viability, and since drugs are chemicals, PGC-D and PGC-Glu-D exhibited lower cell viability, depending on their concentration.[27] Cell viability greater than 80% indicates non-toxicity to cells, cell viability between 60% and 80% suggests slight toxicity, cell viability between 40% and 60% indicates moderate toxicity, and cell viability below 40% indicates severe toxicity.[28] Therefore, PGC-blank, PGC-Glu-blank, and PGC-Glu-D (91.00 ± 4.82, 93.22 ± 5.94, and 84.53 ± 12.33 %, respectively) are non-toxic to cells, while PGC-D (76.25 ± 17.80 %) has slight toxicity.

Table 5 Moisture uptake properties of hydrocolloid films and cytotoxicity.

Sample	Moisture Uptake (%)		Cytotoxicity	
	Day 2	Day 6	Cytotoxicity (%)	Cell viability (%)
PGC-blank	29.43 ± 1.42[a]	34.61 ± 1.40[a]	9.00 ± 4.82[a]	91.00 ± 4.82[a]
PGC-Glu-blank	30.67 ± 5.56[a]	34.29 ± 0.44[a]	6.78 ± 5.94[a]	93.22 ± 5.94[a]
PGC-D	29.96 ± 0.52[a]	36.82 ± 0.37[b]	23.75 ± 17.80[a]	76.25 ± 17.80[b]
PGC-Glu-D	29.88 ± 1.94[a]	36.21 ± 1.67[b]	15.47 ± 12.33[a]	84.53 ± 12.33[b]

Average values with a different letter ("a", "b") show significant difference between each sample. In contrast, average values with the same letter are not statistically different ($p < 0.05$) according to Tukey's test.

The hydrocolloid films are flexible and translucent, with a thickness between 0.28 ± 0.05 and 0.38 ± 0.05 mm, a pH range suitable for split-thickness skin graft donor sites. The water vapor transmission rate (WVTR) was in inappropriate ranges, and the flow pattern of the solution was pseudoplastic. Furthermore, adding glutaraldehyde to the formulation causes the hydrocolloid film to turn yellow. It requires more force to break, while the addition of the drug cocktail into the hydrocolloid film lowers the pH value, reduces the integrity of the hydrocolloid film, shortens the dissolution time, and increases moisture uptake, which are good properties for topical wound dressings for split-thickness skin graft donor sites.

Conclusions

Hydrocolloid films can be prepared from LMP, SCMC, and gelatin using the casting technique. The resulting hydrocolloid films have desirable physical and functional properties as topical wound dressings for split-thickness skin graft donor sites. In summary, both hydrocolloid formulations, with and without glutaraldehyde, exhibit appropriate physical properties for use as topical wound dressing materials for skin grafts, with the choice depending on the desired stability at the application site. Additional recommendations for this research include analyzing the quantities of other active ingredients in the formulation and conducting Fourier Transform Infrared (FTIR) spectroscopy to determine the compatibility and interactions between the active ingredients and the polymer. Furthermore, the release of each drug from the hydrocolloid film should undergo further testing, and safety should be evaluated in volunteers before use in patients.

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