Research Article

Fabrication and Characterization of Polylactic Acid (PLA) Microporous Film Coated with Gelatin and *Chromolaena Odorate* Leaf Extract for Wound Dressing Application

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Abstract
The infection of the skin wound is the cause of pain and difficult recovery. This work has developed wound dressing from microporous PLA film-coated gelatin from marrow bone and jellyfish with blended *Chromolaena odorata* leaf extract at low concentration to stimulate skin cell growth. The microporous PLA film was produced by solution method with different ratios between dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) at 25:35, 25:50, and 25:65, respectively. The optical microscope and scanning electron microscope (SEM) were used to analyze the pore size of microporous PLA film and its physical characteristics after coating with different gelatins by dip coating process. The wound dressing film was determined for cell viability after testing with NIH/3T3 and HaCaT in the culture cell plate. The testing method was followed by a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay to measure cell stimulation and cell viability using a UV absorption technique after staining. The result shows that our wound dressing can stimulate both NIH/3T3 and HaCaT keratinocytes but with different characteristics due to the different releasing characters of gelatins and different responses of cell types depending on *C. odorata* leaf extract concentration. The microporous PLA film-coated gelatin from jellyfish revealed better skin cell growth in both NIH/3T3 and HaCaT keratinocytes than that of coated gelatin from marrow bone because of the continuous releasing rate after contact with water of gelatin from jellyfish. Moreover, *C. odorata* leaf extract which was used for reducing the inflammation of the cellular injury displayed toxic HaCaT keratinocyte at high concentrations but it did not affect the NIH/3T3 toxicity. Interestingly, it can stimulate the cell viability of NIH/3T3 until 3 mg/mL of *C. odorata* leaf extract concentration and displays constant cell viability afterward.

Keywords: Cell viability, *Chromolaena odorata* leaf extract, Gelatin, PLA film, Wound dressing

1 Introduction

Wound dressing technology has been developed to reduce the healing period and release the patient's pain. It is not only to prevent a wound from bacteria or any contamination from the environment and absorb the exudate from the wound but also to keep the wound in the best condition for symptom control and stimulate the wound's healing [1]–[4]. The wound-healing process includes inflammation, proliferation, and maturation [2], [3]. To facilitate this, the dressing should keep the moisture of the wound, absorb the excess exudate, maintain a temperature similar to the body, reduce scar tissue during the healing process, transmit the gas and moisture between the wound and environment, prevent

infection and minimize the pain during healing or after removing wound dressing [4]. Moreover, smart wound dressing that could encapsulate the drug and release it at specific conditions such as pH, thermal, and light trigger would be presented [5]. There are many technologies of fabricated wound dressing, such as hydrocolloid, wound exudate, hydrogel, foam, film, etc. [1]–[4], [6], [7]. However, there is no dressing that can cover all those factors to heal the wound. Thus, wound dressing should be managed by considering the characteristics of the wound and its advantages. For example, radiation therapy burns that the skin side effect from radiation causes a decrease in cell proliferation and collagen activity suitable for film dressing [3]. Our research focuses on the film dressing technique because of its easy fabrication and low cost.

Wound dressing materials can be divided into two main categories including natural and synthetic materials. For natural materials, animal collagen is the best match for wound healing but the cost of it is a crucial factor for being used [8]. Therefore, gelatin, the fibrous protein derived from collagen by acid/alkaline denaturation that can be partially denatured by heating under acidic or alkaline conditions, is an interesting material [9]. Gelatin has been widely used in the food, biomedical, and cosmetic industries and offers significant advantages over alternative gelling agents [10]. Moreover, due to its great biocompatibility and biodegradability, gelatin is frequently utilized in biomedicine [11] and healing wounds [12], [13]. It can be used as a sponge or film for skin tissue engineering and stimulate skin cells during wound healing [3]. Gelatin for medical application in commercial grade comes from the marrow bone of mammal or marine skin, such as tilapia fish. Marine gelatin is increasingly used because of its similarities to mammalian gelatins, as well as to bovine and porcine gelatin. Besides gelatin derived from marine organisms, jellyfish is attractive as a potential source of substances for use in biotechnology due to their inexpensive source [14]. Jellyfish have many uses, including serving as a source of valuable molecules like collagen, gelatin, and protein hydrolysates, as well as providing high-protein food [15]. In addition, it is rich in the collagen protein that is the precursor for derived gelatin that has great biocompatibility with human collagen [16], [17]. It has been used in biomedical applications such as scaffolds for tissue growth and regeneration [12], [18], [19].

The other natural material is derived from plants or herbs such as cotton, natural fiber, Aloe vera, etc. The medicinal plant in Thailand, namely siam weed (*Chromolaena odorata Linn.*), is a traditional medicinal plant known for its therapeutic effects on the body. It has been demonstrated that *C. odorata* leaf extract possesses numerous ethnopharmacological properties, including antibacterial, analgesic, antioxidant, wound healing, and anti-inflammatory effects [20]–[22]. Recently, Latif *et al.*, [23] investigated the wound-healing activity of a *C. odorata* layered-nitrile rubber transdermal patch on excision wound healing in rats. Interestingly, the results obtained found that the *C. odorata* layered-nitrile rubber transdermal patch was effective in healing skin wounds. Another type of wound dressing material is a synthetic material that is mostly polymeric material. The most interesting used polymers are polyurethane, nylon, polyvinyl alcohol, and polycaprolactone, which are synthesized from petrochemicals [8], [24]. Biopolymer is another synthetic material that is interested in wound dressing applications due to biocompatibility with the cell and stimulating cell proliferation rate. Polylactic acid (PLA) is one of the interesting biopolymers produced from plants, which is a biocompatible material that is widely used in medical applications [25], [26]. Chen *et al.*, [27] reviewed and summarized from previous research that skin cells could adhere and proliferate on PLA-based materials with low cytotoxicity and infection. Many researchers [27]–[33] studied on electrospinning process of PLA fibrous material and adding gelatin or drugs in PLA-based materials for wound healing dressing. These researchers found a high surface area of electrospun fibrous materials and a small amount of gelatin blending improving the skin wound healing process because it controlled water and moisture loss, and enhanced cell adhesion and cell proliferation. However, the electrospinning process is limited for fabrication because it is difficult to control the uniform fiber formation, which is related to the viscosity of the solution. Thus, our work used the solution process and drying solvent under room temperature to form microporous PLA film [34] and used the pore size of PLA film for encapsulating substances. This technique of encapsulating gelatin and *C. odorata* leaf extract by dip coating has low cost and is easy to scale up for further use and there is no research using these compositions and fabrication process as a wound dressing from the best of our knowledge.

In this work, PLA microporous film with various solvent ratios between dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) was studied to obtain the smallest and most homogeneous pore size of microporous film. Then, the PLA microporous film was dip-coated with gelatin from marrow bone and jellyfish with or without *C. odorata* leaf extract. The film was observed for its morphology and tested cell viability of NIH/3T3 and HaCaT keratinocyte in cell culture medium by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. In addition, the accumulative releasing rate of different gelatins was investigated to identify the character of gelatin to release the important substance, namely *C. odorata* leaf extract, during healing wounds. The objective of this work is to develop the wound dressing by choosing PLA as a medium because it is a biopolymer that is biocompatible with the cell and can be biodegradable without toxicity while gelatin is selected because it has found numerous applications in tissue engineering due to its cell adhesion and wound healing properties, as well as its biodegradability, biocompatibility, water retention capacity, and film-forming ability [9], [11], [19], [31], [35]. In addition, *C. odorata* leaf extract is chosen because it has numerous ethnopharmacological properties that might be helpful for wound healing applications.

2 Materials and Methods

2.1 *Materials*

Polylactic acid (PLA, 4032D, blown film grade with L-lactide content of 98%, an average molecular weight of 111 kDa, and a density of 1.24 g/cm^3), tetrahydrofuran (THF, Reagent grade) and dichloromethane (CH_2Cl_2, HPLC grade) were purchased from NatureWorks, USA, Merck, Germany, and Honeywell Burdick & Jackson, Singapore. Gelatin from marrow bone (Pharmaceutical grade type B, 180 bloom, 7 mesh, 95% purity, and moisture below 16%, dissolved in warm water at around 80°C) was supported by Cartino Gelatin Co., Ltd, Thailand. Gelatin from jellyfish (258.6 bloom, type A) was synthesized from the umbellar part by hydrolysis method.

2.2 *Preparation and extraction of C. odorata leaf*

Siam weed (*C. odorata*) leaves were washed and cleaned. They were then desiccated (dried) at room temperature until almost dry. The leaves were subsequently placed in a hot air oven at 60 °C to remove any remaining moisture. The dried leaves were then ground using a mechanical grinder (Grinder, Spring green evolution speeds 32000 rpm). After that, 200 g of the finely ground powder was weighed and extracted with 800 ml of distilled water that was heated to 90 °C for 3–4 h. The extract was filtered first through the Whatman No. 4 filter paper. Finally, the extract was then freeze-dried and kept at 4 °C until use. The yield of *C. odorata* leaf extraction was around 10.53%wt

2.3 *Gelatin extraction from jellyfish*

Gelatin extracted from the salted umbellar part of jellyfish (*Lobonema smithii*). They are washed and cleaned several times to remove salt, and the salt content is measured using a refractometer until no salt content. Then 0.5 M NaOH (1:3, w/v) was used to remove the residual by shaking at 150 rounds per minute and 4 °C for 2 h, washing three times, and adjusting pH with 0.2 M HCl (1:2, w/v) at 150 rounds per minute for 1 h at 25 °C. Then, rinse the sample until the pH is neutral, extract the sample with distilled water (1:2, w/v) at 60 °C for 48 h in a water bath, and filter the sample using the Whatman No. 4 filter paper. Finally, the gelatin solution was dried in a hot air oven at 70 °C and grounded in a fine particle before dissolving again in DI water to control the gelatin concentration. The yield of gelatin extraction from jellyfish was around 1.5%wt

2.4 *Microporous PLA film forming by solvent method*

PLA pellet was grounded into a small piece by a mechanical grinder (Grinder, Spring green evolution speeds 32000 rpm). 1 g of PLA powder was dissolved in 25 mL of CH_2Cl_2 (0.04 g/mL of PLA in CH_2Cl_2) by a magnetic stirrer. Then, THF at 35, 50, and 65 mL was mixed with a polymer solution (0.017, 0.013, 0.011 g/mL of PLA in mixture solution) and stirred continuously for an hour to homogenize THF in the polymer solution. The exact ratios of dichloromethane and tetrahydrofuran were 25:35, 25:50, and 25:65 respectively. The homogeneous polymer solution was poured into a 16×145 mm Petri dish and evaporated in a hood at room temperature to form a microporous PLA film at different ratios between CH_2Cl_2 and THF.

2.5 *Dip coating process of gelatins and their blend with C. odorata leaf extract on PLA microporous film*

Gelatin from marrow bone or jellyfish around 2.5 g was dissolved in 10 mL of DI water for gelatin without *C. odorata* leaf extract. If *C. odorata* leaf extract in aqueous solution (20 mg/mL of extract) was added into gelatin solution at 1, 3, and 5 mg/mL of gelation solution, the DI water was adjusted to the same concentration of gelation solution at 0.25 g/mL. For example, if we added *C. odorata* leaf extract in an aqueous solution of 0.5 ml, the DI water was reduced to 9.5 mL to maintain the total water in our solution at 10 mL for 1 mg/mL of *C. odorata* leaf extract and added 2.5 g of gelatin powder. The solution was stirred at 60 °C with a magnetic stirrer on a hotplate stirrer until completely dissolved gelatin in water. PLA microporous film with the smallest pore size from the previous experiment was dipped in gelation solution to form a thin film coated on PLA microporous film as an active ingredient for stimulating skin cell growth. The film was dipped in the solution for a short period (1–2 s) and placed on the screen. The film was then dried in the natural air at room temperature overnight by keeping it in the hood. Then, it was cut into a circular shape with a paper punch, sterilized under UVC for an hour, and kept in an aluminum foil for hygiene.

2.6 *Cell viability assessment of human keratinocyte (HaCaT) and mouse fibroblast (NIH/3T3) by MTT assay and morphological observation*

Cell viability measurement by MTT assay is performed in 96-well plates with a density of HaCaT cells of 1×10^4 cells/well and NIH/3T3 fibroblast cells of 5×10^3 cells/well were cultured in Dullbecco's modified eagle medium (DMEM, Gibco, USA) containing 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin and 25 µg/mg amphotericin B). Then, it was incubated in a CO_2 incubator with 5% CO_2 at 37 °C for 24 h. For sample preparation, the films that underwent UV irradiation sterilization were placed into each well of cell culture by divided into six groups including untreated (no film, used as a control), PLA microporous film, PLA microporous film coated with gelatin from marrow bone, PLA microporous film coated with gelatin from jellyfish, PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 1, 3, 5 mg/mL of gelation solution and PLA microporous film coated with gelatin from jellyfish blended with *C. odorata* leaf extract at 1 mg/mL of gelatin solution. After that, it was further incubated in a CO_2 incubator with 5% CO_2 at 37 °C for 24 h. After incubation, the morphological observations of cells were visualized and captured using an inverted microscope (EVOS M5000, Thermo Fisher Scientific, USA). Next, cell viability was assessed by removing the cell culture media from the 96-well plates and replaced with the solution of 1x PBS containing MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at 0.5 mg/mL of concentration, incubated in a CO_2 incubator with 5% CO_2 at 37 °C for an hour. Subsequently, the formazan salt was dissolved using 100% DMSO at a volume of 150 µL per well, and the solution was thoroughly mixed. Absorbance was measured at a wavelength of 570 nm using a microplate reader (Molecular Devices Spectramax M2, Avantor, USA) to calculate the cell viability of the treated films compared to the untreated control group, expressed as a percentage of control. The group receiving only DMEM was considered to have a cell viability of 100%.

2.7 *Releasing rate of gelatin in aqueous solution by Bradford assay*

Gelatins from marrow bond and jellyfish powder were dissolved in DI water and poured into the 36-well plate to form a cylindrical shape of gelatin. The solution was dried in a hot air oven at 70 °C for 3–4 days until completely dry. The cylindrical shape specimens of gelatins from marrow bone and jellyfish were measured in the surface area of each specimen before testing in 50 mL of DI water. Sample water was measured for the protein content after soaking in DI water at 1, 2, 3, and 4 hours by adding Bradford reagent. Finally, the sample was incubated for 5–10 min at room temperature and measured the protein content using a UV / Vis Spectrophotometer (Genesys 10s UV-Vis, Thermo Fisher Scientific, USA) at 595 nm of absorbance.

2.8 *Characterization*

PLA microporous film and PLA microporous film coated with gelatin of different types including gelatin from marrow bone and jellyfish were observed microstructure by optical microscope (ZEISS, Axiolab 5,

Germany) and scanning electron microscope (LEO, LEO1450VP, Germany) at 15 kV of accelerating voltage.

3 Results and Discussion

3.1 *Morphology of PLA film before and after being coated with gelatin*

PLA microporous film at different ratios of CH_2Cl_2:THF after drying the solvent in the hood was investigated the microstructure in terms of pore size because the pore size affects the cell growth and ability to encapsulate the gelatin into the pore for release during contact with the cell. The results of PLA microporous film from an optical microscope (OM) and scanning electron microscope (SEM) are shown in Figure 1. It has been found that the best ratio of CH_2Cl_2:THF to form the homogenous and smallest pore size in this work is 25: 50 due to the appropriate ratio of these solvents. This ratio was used to fabricate the PLA microporous film of this work. Then, PLA microporous film was coated with gelatin and blended with *C. odorata* leaf extract at different concentrations of 1, 3, and 5 mg/mL in gelatin solution by dip coating process. The results from OM and SEM in Figure 2 revealed that coated PLA microporous film with gelatin from marrow bone exhibited a smooth surface compared to gelatin from jellyfish. This might be the character of gelatin from jellyfish after the hydrolysis process and dried in an oven before use to control the same concentration of gelatin at 0.25 g/mL of each formula. The bloom strength of extracted gelatin from jellyfish after testing by following ISO 9665 standard was around 258.6 while the bloom strength of gelatin from marrow bone was 180 from the specification which meant the gelatin from jellyfish had higher gelatin molecular weight than those of gelatin from marrow bone. The higher bloom strength of gelatin influenced the higher stiffness character of gelatin after fabrication [36]. Thus, this might be the reason why the rough surface of PLA microporous film coated with gelatin from jellyfish was obtained in this work. Moreover, after blending the gelatin with *C. odorata* leaf extract at 1 mg/mL, the morphology of the film was unchanged when compared with the film without blending with *C. odorata* leaf extract both on gelatin from marrow bone and jellyfish.

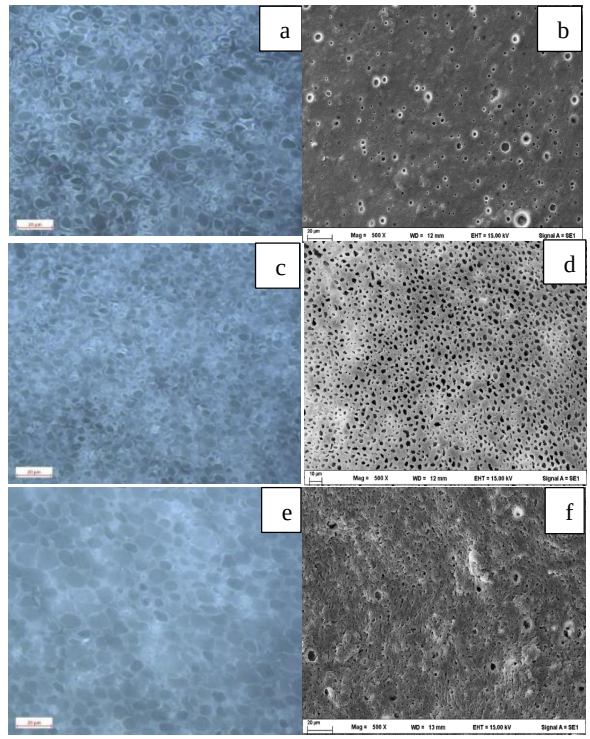

Figure 1: Morphology of PLA microporous film at different ratios of CH_2Cl_2:THF; (a) OM, (b) SEM of PLA film of CH_2Cl_2:THF = 25:35, (c) OM, (d) SEM of PLA film of CH_2Cl_2:THF = 25:50, (e) OM, (f) SEM of PLA film of CH_2Cl_2:THF = 25:65.

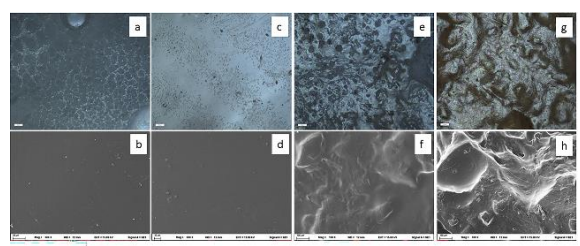

Figure 2: Morphology of PLA microporous film coated with gelatin from marrow bone; (a) OM and (b) SEM, PLA microporous film coated with gelatin from marrow bone blended with *C. odorata leaf* extract; (c) OM and (d) SEM, PLA microporous film coated with gelatin from jellyfish; (e) OM and (f) SEM, PLA microporous film coated with gelatin from jellyfish blended with *C. odorata* leaf extract at 1 mg/mL; (g) OM and (h) SEM.

3.2 *Effect of PLA microporous film and their coated with different gelatins from marrow bone and jellyfish on human keratinocytes HaCaT and mouse fibroblast NIH/3T3 cells*

PLA microporous film, PLA microporous film coated with gelatin from marrow bone, and PLA microporous film coated with gelatin from jellyfish were tested for cell viability of HaCaT and NIH/3T3 cells. The morphology of HaCaT and NIH/3T3 cells observed after the test were obtained in Figures 3 and 4 respectively. The cells after being tested with these films including PLA microporous film, PLA microporous film coated with gelatin from marrow bone, and PLA microporous film coated with gelatin from jellyfish had nontoxicity compared with untreated cells which was a control that did not submerge the film into the culture medium during the test. These results were the same in both on HaCaT and NIH/3T3 cells observation. This might be due to the compatibility of PLA film and gelatin on cells which were nontoxic materials for cells but all PLA microporous films as well as with and without coated with gelatin from marrow bone and jellyfish did not stimulate cell growth both on HaCaT and NIH/3T3 cells after testing by MTT assay and calculated cell viability percent in Figure 5. The cell viability did not significantly change when compared with untreated cells (100 ± 13.62, 96.94 ± 15.40, 96.56 ± 15.04, 96.56 ± 7.12 of untreated, PLA, PLA/MB, PLA/JF for HaCaT cell viability percent (Figure 5(a)) and 100 ± 8.10, 103.30 ± 3.61, 107.90 ± 11.10, 102.07 ± 12.07 of untreated, PLA, PLA/MB, PLA/JF for NIH/3T3 cell viability percent (Figure 5(b)). For this reason, PLA microporous films coated with gelatin from marrow bone and gelatin from jellyfish were used as a control to compare the effect of *C. odorata* leaf extract on morphology and cell viability of HaCaT and NIH/3T3 cells. Similarly, the fabrication of composite sponge using curcumin-loaded chitosan/gelatin for wound healing applications. Their cell viability tests suggest that these sponges have generally low cytotoxicity to L929 fibroblast cells [37].

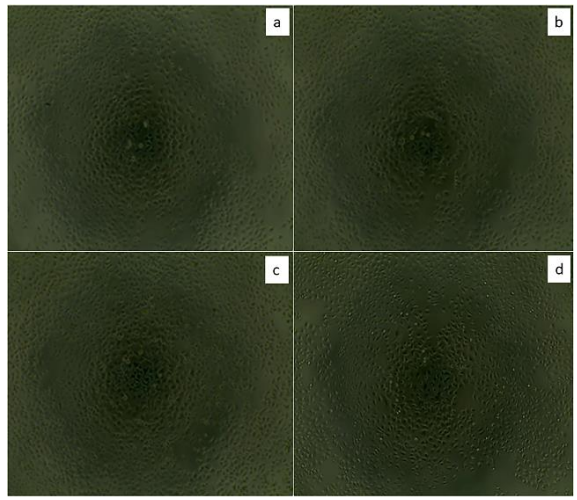

Figure 3: The images of HaCaT cell tested by MTT assay of (a) untreated cell, (b) treated with PLA microporous film, (c) treated with PLA microporous film coated with gelatin from marrow bone, (d) treated with PLA microporous film coated with gelatin from jellyfish.

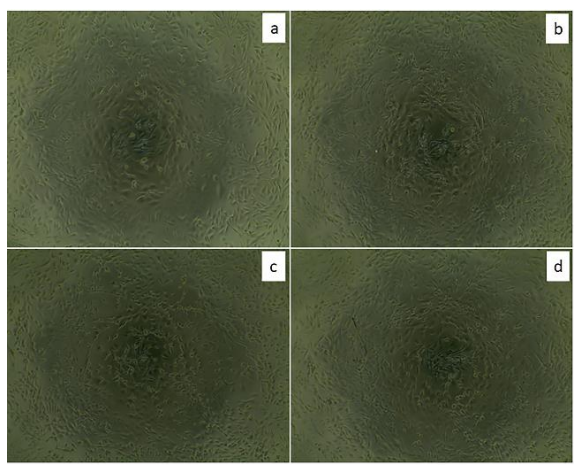

Figure 4: The images of NIH/3T3 cells tested by MTT assay of (a) untreated cell, (b) treated with PLA microporous film, (c) treated with PLA microporous film coated with gelatin from marrow bone, (d) treated with PLA microporous film coated with gelatin from jellyfish.

3.3 *Effect of C. odorata (L.) leaf extract concentration in gelatins on morphology and cell viability of HaCaT and mouse fibroblast NIH/3T3 cells*

PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 1, 3, and 5 mg/mL in gelation solution was determined for the morphology and cell viability of HaCaT and NIH/3T3 cells. Morphological properties of HaCaT and NIH/3T3 cells after testing are shown in Figures 6 and 7 respectively. Figure 6 is the image of the HaCaT cell tested by MTT assay which shows the same cell growth after testing when varying the concentration of *C. odorata* leaf extract from 0–5 mg/mL in gelatin solution. This result is related to the result of cell viability percent after calculation in Figure 8, which shows slightly changed cell viability percent of HaCaT keratinocytes at different concentrations of *C. odorata* leaf extract in gelatin from marrow bone. The trend is slightly toxic for HaCaT cells if the high concentration of *C. odorata* leaf extract in gelatin is blended. Interestingly, the result of NIH/3T3 cells, when tested with PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at different concentrations, exhibits inversely in Figure 7. The morphology shows that the high cell growth after testing of NIH/3T3 is observed at a high concentration of *C. odorata* leaf extract in gelatin from marrow bone when compared with PLA microporous film coated with gelatin from marrow bone without blended with *C. odorata* leaf extract. The yellow spot in Figure 7 is the death cell observed from the optical microscope after testing, which confirmed that Figure 7(b)–(d) has less yellow spot than Figure 7(a). These results are also related to the result of cell viability percent of NIH/3T3 cells after calculation in Figure 8. PLA film coated with gelatin from marrow bone was used as a control and set as 100% cell viability because the result of cell viability (Figures 3, 4 and 5) of untreated (no film), treated with PLA microporous, treated with PLA microporous film coated with gelatin from marrow bone, treated with PLA microporous film coated with gelatin from jellyfish were not a significant change. Thus, we can use PLA film coated with gelatin from marrow bone as a control as same as using untreated (no film) or treated with PLA microporous film.

The result of cell viability percent of NIH/3T3 cells in Figure 8 found that the amount of *C. odorata* leaf extract in gelatin from marrow bone can stimulate cell growth if compared with PLA microporous coated with gelatin from marrow bone without blended with *C. odorata* leaf extract. In addition, the higher concentration of *C. odorata* leaf extract in gelatin from marrow bone did not affect the cell growth of NIH/3T3 cells after the calculation of cell viability percent (100 ± 11.59, 102.57 ± 7.72, 101.31 ± 7.56, 95.92 ± 6.98 of PLA/MB, PLA/MB-C1, PLA/MB-C3, PLA/MB-C5 for HaCaT cell viability percent (Figure 8(a)) and 100 ± 6.57, 117.85 ± 8.25, 120.84 ± 10.59, 121.70 ± 13.38 of PLA/MB, PLA/MB-C1, PLA/MB-C3, PLA/MB-C5 for NIH/3T3 cell viability percent (Figure 8(b)). The result could summarize that the amount of *C. odorata* leaf extract in gelatin from marrow bone did not affect the cell growth of HaCaT cells but a small amount of its blended in gelatin from marrow bone could stimulate the cell growth of NIH/3T3 cells. From this result, the amount of *C. odorata* leaf extract in gelatin solution was set at 1 mg/mL to compare the effect of different types of gelatin, which were gelatin from marrow bone and jellyfish that blended with *C. odorata* leaf extract on morphology and cell viability of HaCaT and NIH/3T3 cells. These results showed the proliferation of fibroblast cells after being added to PLA/gelatin film, supporting scientific studies that revealed *C. odorata* extracts exhibit several activities involved in wound healing [38]. Additionally, several studies have reported that its phytochemical is capable of exhibiting a wide spectrum of pharmacological activities, including antioxidant activity, hypoglycemic and hypocholesterolemic effects in animals [39], as well as stimulating cell migration and proliferation, which are crucial for wound healing [20], [21]. Furthermore, the study by Latif *et al.*, [23] proved that the *C. odorata* layered-nitrile rubber transdermal patch was effective in healing skin wounds.

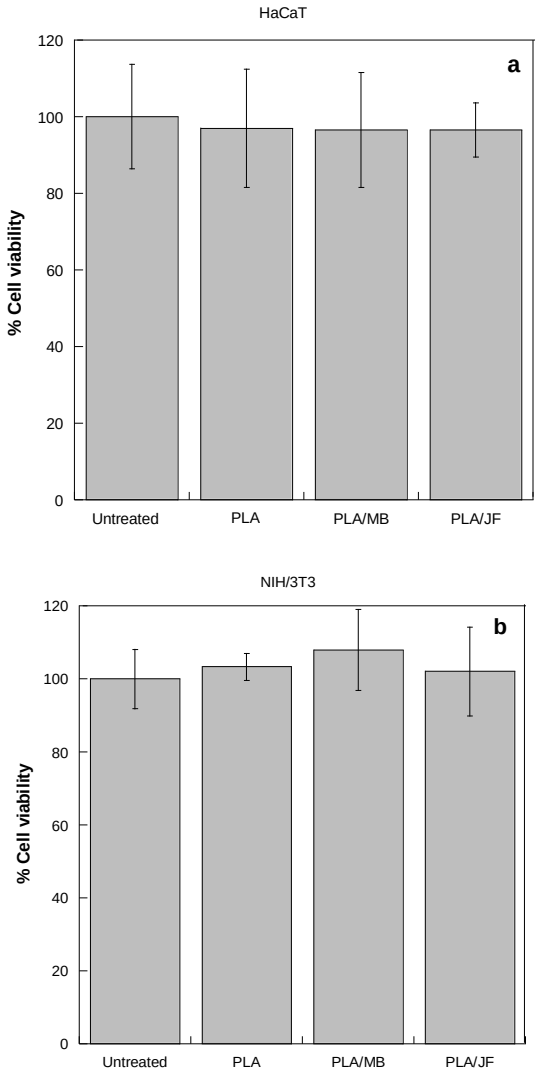

Figure 5: Cell viability result of untreated, treated with PLA microporous, treated with PLA microporous film coated with gelatin from marrow bone, treated with PLA microporous film coated with gelatin from jellyfish tested by MTT assay of (a) HaCaT and (b) NIH/3T3 cells.

PLA microporous films coated with gelatin from jellyfish with and without *C. odorata* leaf extract at 1 mg/mL of gelatin solution was measured for the cell viability of HaCaT and NIH/3T3 cells to compare the influence of the amount of *C. odorata* leaf extract in gelatin from jellyfish. Figures 9(a) and (b) show the effect of with and without *C. odorata* leaf extract in gelatin from jellyfish on cell viability of HaCaT and Figure 9(c) and (d) display the effect of with and without *C. odorata* leaf extract in gelatin from jellyfish on cell viability of NIH/3T3 cells respectively. Morphology of cell growth after testing by MTT assay revealed the same both on HaCaT and NIH/3T3 cells after using PLA microporous films coated with gelatin from jellyfish with and without *C. odorata* leaf extract at 1 mg/mL of gelatin solution. The cell can be stimulated by these PLA microporous films without any toxicity. Moreover, the cell viability of PLA microporous film coated with gelatin from marrow bone and jellyfish at 1 mg/mL of *C. odorata* leaf extract in gelatin solution on HaCaT and NIH/3T3 cells is calculated and compared in Figure 10 (102.67 ± 7.72, 111.12 ± 13.93, 117.85 ± 8.25, 119.72 ± 11.10 of PLA/MB-C1, PLA/JF-C1 of HaCaT cell viability percent and NIH/3T3 respectively). The result shows that PLA microporous film coated with gelatin from jellyfish at 1 mg/mL of *C. odorata* leaf extract in gelatin solution has better cell viability than PLA microporous film coated with gelatin from marrow bone at 1 mg/mL of *C. odorata* leaf extract in gelatin solution both on HaCaT and NIH/3T3 cells. Our assumption is the fast releasing of gelatin from jellyfish after testing in a culture medium might stimulate more than gelatin from marrow bone. To explore this assumption, the experiment of the releasing rate of gelation in DI water was explored in this research. This observation was supported by the research conducted by Lueyot *et al.*, [40], which reported that the gel strength of jellyfish gelatin is lower than that of bovine and fish gelatin. This difference may be attributed to variations in collagen type, amino acid composition, α-chain to β-chain ratio, and α-chain peptides, potentially allowing it to release more than marrow bone gelatin. Moreover, it has been demonstrated that jellyfish collagen is non-toxic, and maintains a higher cell viability of osteoblasts and fibroblasts compared to bovine collagen [16], [35], [41].

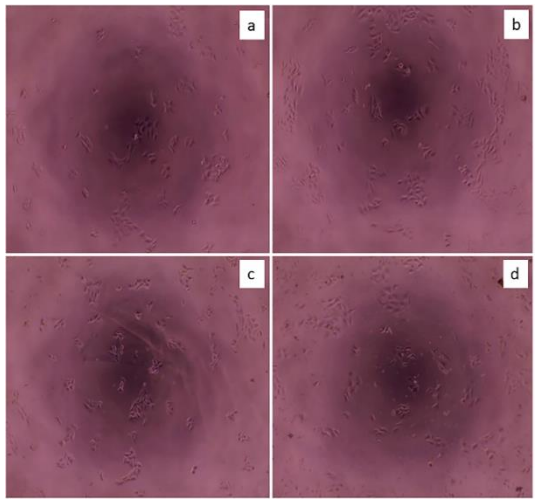

Figure 6: The images of HaCaT cell tested by MTT assay of (a) PLA microporous film coated with gelatin from marrow bone, (b) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 1 mg/mL, (c) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 3 mg/mL, (d) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 5 mg/mL.

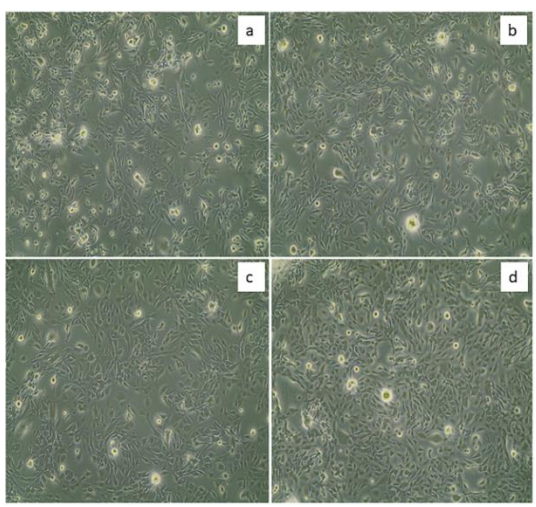

Figure 7: The images of NIH/3T3 cells tested by MTT assay of (a) PLA microporous film coated with gelatin from marrow bone, (b) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 1 mg/mL, (c) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 3 mg/mL, (d) PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at 5 mg/mL.

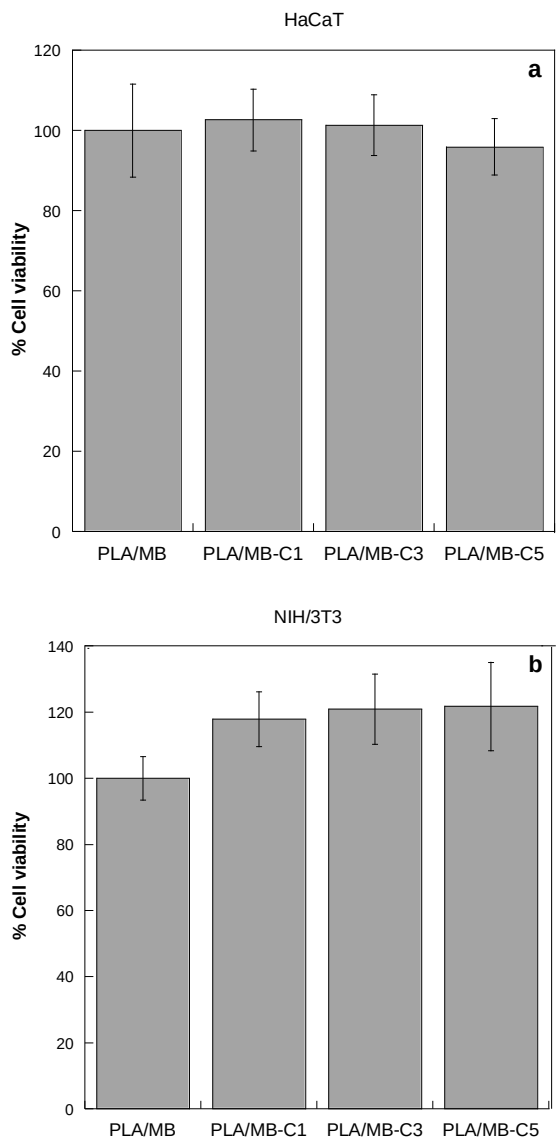

Figure 8: Cell viability result of PLA microporous film coated with gelatin from marrow bone blended with different concentrations of *C. odorata* leaf extract (1, 3, 5 mg/mL) that tested by MTT assay of (a) HaCaT and (b) NIH/3T3 cells.

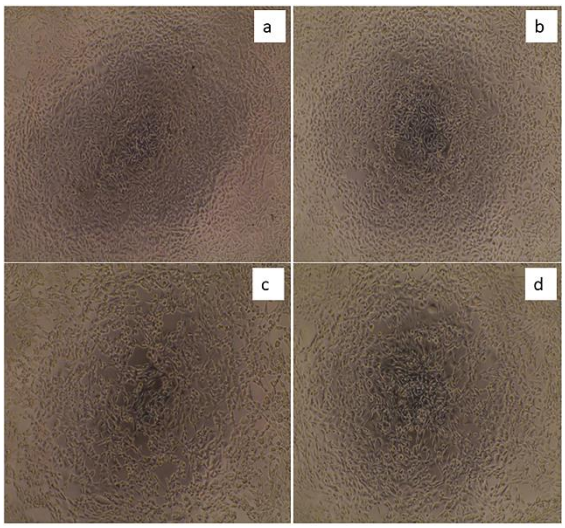

Figure 9: The image of HaCaT keratinocytes tested by MTT assay of (a) PLA microporous film coated with gelatin from jellyfish, (b) PLA microporous film coated with gelatin from jellyfish blended with *C. odorata* leaf extract at 1 mg/mL, NIH/3T3 cells tested by MTT assay of (c) PLA microporous film coated with gelatin from jellyfish, (d) PLA microporous film coated with gelatin from jellyfish blended with *C. odorata* leaf extract at 1 mg/mL.

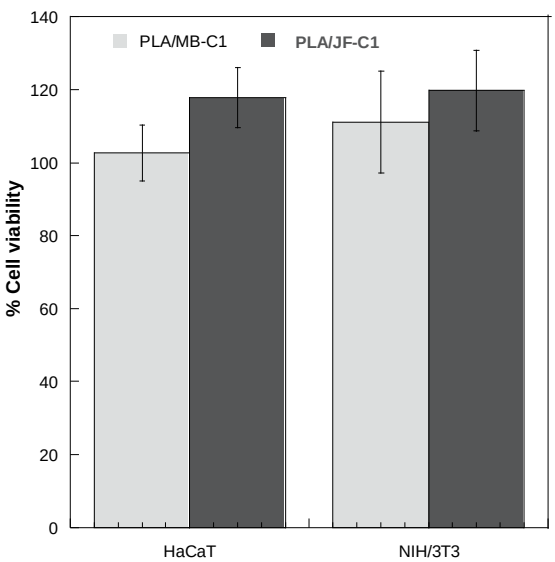

Figure 10: Cell viability of HaCaT and NIH/3T3 cells results after testing with PLA microporous film coated with gelatin from marrow bone and jellyfish blended with *C. odorata* leaf extract at 1 mg/mL of concentration.

3.4 *Releasing rate of gelatins in DI water from detecting protein concentration by Bradford assay*

Gelatins from marrow bone and jellyfish were fabricated as a cylindrical shape in a 24-well plate and calculated the surface area of each sample to collect the surface area after calculating releasing rate of gelatin in DI water. Three pieces of each type of gelatins from marrow bone and jellyfish were soaked in DI water and sampled every hour for 4 h. The time of release rate was set at 4 h because the complete dissolving of gelatin coated on PLA microporous film in DI water could be clearly observed within 4 h after the preliminary test. The accumulative releasing rate of gelatin from marrow bone and jellyfish in Figure 11 displays different characteristics which might be the reason why gelatin from jellyfish coated on PLA microporous film has high cell stimulation. This result was obtained by Bradford assay to detect the protein content in DI water after sampling for calculating the accumulative releasing rate of gelatin due to the protein nature of gelatin. The accumulative releasing rates per specific surface area of gelatin from marrow bone were 0.14 ± 0.002, 0.17 ± 0.035, 0.14 ± 0.002, 0.16 ± 0.035 μg/mL.mm^2 at 1, 2, 3 and 4 h of sampling time while the accumulative releasing rate per specific surface area of gelatin from jellyfish were 0.12 ± 0.049, 0.18 ± 0.022, 0.39 ± 0.206, 0.43 ± 0.169 μg/mL.mm^2 at 1, 2, 3 and 4 h of sampling time. From the result, the accumulative releasing rate after collecting the surface area of gelatin from marrow bone is steady during sampling while the accumulative releasing rate of gelatin from jellyfish shows different results. The accumulative releasing rate of gelatin from jellyfish after collecting the surface area of gelatin increased after soaking in DI water for a long period. This might be the reason why gelatin from jellyfish can better stimulate the cell growth both on HaCaT and NIH/3T3 cells than that of gelatin from marrow bone when it is coated on PLA microporous film. In our view, the continuous releasing rate of gelatin from jellyfish during testing the cell might affect the releasing rate of *C. odorata* leaf extract in gelation solution resulting in better cell growth in culture medium. Due to the dissimilarities in gel strength and amino acid composition between jellyfish gelatin and bone marrow gelatin, the release rate may also differ [40]. The supporting finding, recently it was reported that the cumulative percentage release (CPR) of cefazolin from jellyfish hydrogels was higher than that from bovine hydrogels [11].

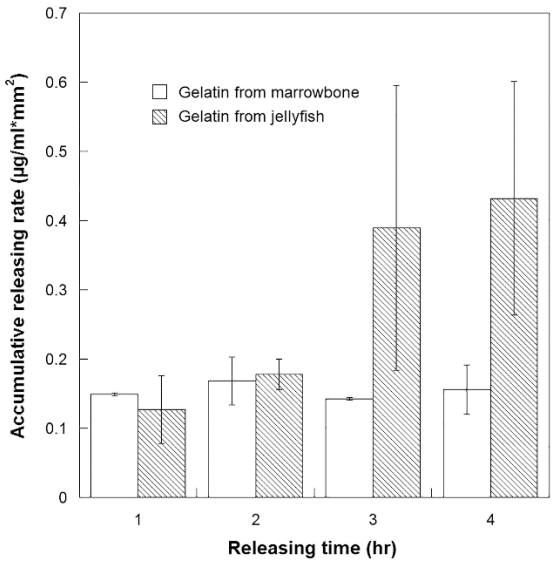

Figure 11: Accumulative releasing rate of gelatin in DI water at different periods.

From the result of different kinds of gelatins that coated on PLA microporous film should be summarized that the gelatin from marrow bone and jellyfish did not have toxicity for the cell after the test by MTT assay due to their biocompatible with the cell. However, the increase of *C. odorata* leaf extract concentration displayed different characteristics in HaCaT keratinocytes and mouse fibroblast NIH/3T3 cells. For HaCaT cells, the high amount of *C. odorata* leaf extract trend to slight toxicity while it was not toxic for mouse fibroblast NIH/3T3 cells at high concentration but it might be stimulated the cell at 1 mg/mL of *C. odorata* leaf extract after that it did not better stimulated the cell of mouse fibroblast NIH/3T3. Moreover, the cell can be better stimulated if the type of gelatin is jellyfish with a small amount of *C. odorata* leaf extract due to the continuous releasing rate of gelatin from jellyfish and the synergistic effect of active ingredient from *C. odorata* leaf extract.

4 Conclusions

This research aimed to fabricated PLA microporous film by solution method at different ratios of CH_2Cl_2:THF. The result found that the ratio between CH_2Cl_2 and THF at 25:50 could form the smallest and most homogeneous pore size of PLA microporous film. Then, it was coated with gelatin from marrow bone and jellyfish with and without *C. odorata* leaf extract and tested cell viability and morphology observation on HaCaT and NIH/3T3 cells in culture medium. PLA microporous film coated with only gelatin from marrow bone and jellyfish did not change morphology and cell viability when compared with the control which was the cell test without the film. This result referred to the nontoxic PLA microporous film and gelatins that coated on the film because these materials were compatible with HaCaT and NIH/3T3 cells but they could not stimulate the cell growth after being tested by MTT assay. Moreover, the small amount of *C. odorata* leaf extract in gelatin from marrow bone solution could stimulate the cell growth for NIH/3T3 cells while the morphology and cell viability of HaCaT keratinocytes exhibited unchanged characteristics. In addition, the cell viability of HaCaT and NIH/3T3 cells after testing with PLA microporous film coated with gelatin from jellyfish blended at the small amount of *C. odorata* leaf extract in gelation solution showed better cell growth than PLA microporous film coated with gelatin from marrow bone blended with *C. odorata* leaf extract at the same concentration. These results might be the continuous releasing rate of gelatin from jellyfish when compared with the steady releasing rate of gelatin from marrow bone which might be the continuous releasing of *C. odorata* leaf extract in gelatin from jellyfish. The different characteristics of releasing gelatin in a liquid medium would be the reason for well-cell growth stimulation in our view. However, this work has to be further developed due to the brittleness of PLA materials after fabricating as a microporous film which might not be effective for use as a wound dressing in daily life. It would be developed by changing another biopolymer such as polybutylene succinate (PBS), polybutyrate adipate terephthalate (PBAT), polycaprolactone (PCL), or a blend of these materials to increase the toughness of microporous film for wound dressing application, which is the future work of our group.

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Author Contributions

S.P., M.R., S.C., C.K., P.C., and W.Y.: conception and experimental design; S.P., M.R., S.C., C.K., and P.C.: investigation, data analysis; S.P. and P.C.: writing reviewing and editing. All authors have read and agreed to publish the version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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