Journal of Advanced Research in Applied Sciences and Engineering Technology

Journal homepage:
https://semarakilmu.com.my/journals/index.php/applied_sciences_eng_tech/index
ISSN: 2462-1943

# Development and In-vitro Evaluation of Konjac Glucomannan/Virgin Coconut Oil Based Asymmetric Membrane for Wound Dressing

Hajaratul Najwa Mohamed[1,*], Norazlina Hashim[1], Mohamad Shahairy Fahmi Affandi[1], Meor Najmuddin Meor Suzurudin[1]

[1] Department of Chemical Engineering, Faculty of Engineering, University College TATI, 2400 Kemaman, Terengganu, Malaysia

**ARTICLE INFO**

***Article history:***
Received 29 March 2023
Received in revised form 9 January 2024
Accepted 4 March 2024
Available online 25 June 2024

***Keywords:***
Virgin coconut oil; Konjac glucomannan; Asymmetric membrane; Wound dressing

**ABSTRACT**

Asymmetric membranes have promising features for use in wound dressings. A permeable bottom layer absorbs the wound exudate, whereas the occlusive top layer restricts microbiological penetration and avoids an excessive loss of water. Recently, asymmetric membranes made of konjac glucomannan (KGM), a natural polysaccharide, have been explored as potential wound treatments. However, there are no studies reported regarding the incorporation of other materials into KGM asymmetric membrane. Due to its biocompatibility and antimicrobial properties, virgin coconut oil (VCO) is a good choice for promoting the healing process. In this study, we examine the potential of using KGM asymmetric membrane with different concentration of VCO as wound dressing material. The membrane sample's asymmetric morphology and good thermal stability were both revealed by scanning electron microscopy and thermogravimetric studies, respectively. In addition, biological and fluid handling capacity analysis indicated that the KGM-VCO membrane is biocompatible and able to maintain the ideal moist environment for wound healing.

## 1. Introduction

The effective wound dressing would require extra healing characteristics, in contrast to conventional wound dressing, which merely passively protect the wound. An ideal wound dressing also needs to effectively promote the healing process by creating the ideal microenvironment for healing, guard against secondary infections, removing excess wound exudate, and enabling ongoing tissue reconstruction processes. Moreover, it should be biocompatible, elastic, handling-resistant and non-toxic [1]. The preservation of an ideal moisture environment is one of the desired qualities of a high-performance wound dressing since it is crucial to the wound healing process [2]. A proper moisture level facilitates autolytic debridement and minimizes tissue dehydration. Additionally, the moisture shields the nerve terminals from exposure and dryness, both of which are directly linked to pain. Moisture also keeps cells alive, causing them to secrete growth hormones and multiply [3].

* *Corresponding author.*
*E-mail address: hnajwa@ucttai.edu.my*

https://doi.org/10.37934/araset.47.2.138147

One innovative method for creating high-performance wound dressings is to use so-called asymmetric membranes, which combine an occlusive surface with a porous structure. These membranes have a stratified morphology that resembles the anatomy of healthy skin, and they are able to produce an environment that is conducive to faster wound healing [3]. Asymmetric membranes made of chitosan and polyurethane, for example, have a great capacity to drain exudate while also preventing the invasion of microbes, surpassing the constraints of standard wound dressings [4-10].

Konjac glucomannan (KGM) has attracted significant attention for use in pharmaceutical and medical applications due to its advantageous intrinsic properties. It is a naturally occurring polymer that is extracted from the tubers of the Amorphophallus konjac plant. Due to their affordability, flexibility, and capacity for swelling, KGM-based materials show promise for use as wound dressings [11-13]. On the other hand, virgin coconut oil (VCO) which is extracted from coconut meat is a pure, cold-pressed coconut oil that has not been refined. It is a promising candidate in promoting healing process due to its biocompatibility and antibacterial activities. In coconut oil, medium chain fatty acids like lauric acid make up more than 50% of the fat content (12:0). The best natural source of lauric acid is coconut oil [14]. Few studies have shown that pure VCO tested in vivo enhances the healing process [15-17]. Shiling and colleagues [18] effectively proved that the fatty acids in VCO can suppress the growth of Clostridium difficile in the fight against microorganisms. These interesting properties of VCO may serve as the additional value if incorporated into KGM asymmetric membrane and enhance the performance.

Therefore, in this study, we have proposed novel combination of KGM and VCO to develop asymmetric membrane for effective wound dressing by using casting-freeze method. Thermal studies and scanning electron microscopy (SEM) were employed to assess the membranes' morphology and thermal stability. In order to evaluate the membrane's effectiveness as a wound dressing, fluid-handling tests, as well as microbial penetration experiments, were conducted.

## 2. Methodology

### *2.1 Preparation of the Asymmetric Membranes*

Konjac powder (90% purity) was purchased from CN Lab Nutrition, Asian Group (Shaanxi, China). The asymmetric membrane was formed using the casting-freeze method [19]. KGM was allowed to dissolved in deionized water for 2 hours to produce 3.5 % (w/w) solution. Next, sodium hydroxide (NaOH) which acted as KGM gelling agent was added to the solution until it achieves a final concentration of 0.1 mol/L. Virgin Coconut oil (VCO) solutions containing 1.2% (w/w) Tween-20 were stirred at 500 rpm until the solution became clear. 60 g of KGM-VCO solution was agitated at 500 rpm for 2 hours to create a stable emulsion for the asymmetric membranes. Then, the KGM-VCO emulsion was casted on petri dish with a diameter of 9.5 cm and dried at 30°C for 24 hours. The samples then were frozen for 24 hours at 6°C. Finally, asymmetric membranes samples were thawed and rinsed with ultrapure water. All the samples were kept in the chiller until further analysis.

### *2.2 Structure and Morphology*

Scanning Electron Microscopy (TM3030Plus, Hitachi, Japan) was used to examine the morphology of the membrane samples. Cross-section micrograph was obtained by cutting the sample into $1cm^2$. The analysis was performed with an acceleration voltage 5 kV and magnification from 50X to 500X. Prior to analysis, the samples were gently pressed between filter paper to remove the excess water.

### *2.3 Thermal Analysis*

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to examine the KGM-VCO membranes. Membrane samples (6 mg) were subjected to TGA at temperatures between 25 and 600 degrees Celsius. With a heating rate of 10°C/min, the nitrogen flow rate was 50 mL/min. Overnight, all membrane sample preparations were freeze-dried. In a sealed alumina crucible, samples between the sizes of 5mg and 10mg were scanned while being heated from 10°C to 450°C for DSC. mL.min-1, in a nitrogen atmosphere, with a flow rate of 50 $mL.min^{-1}$.

### *2.4 Fluid Handling Capacity*

According to the BS EN 13726-1 technique for hydrocolloids and dressings [20], the fluid-handling capacity (FHC) of the membrane samples was examined. This test determines the capacity of a membrane to remove exudate by the sum of its swelling capacity and moisture vapor transmission rate (MVTR). Prior to FHC test, all samples were first sterilized with ethanol 70% (v/v) and then rinsed with phosphate buffered saline (PBS). NaCl (142 mmol/L) and $CaCl_2$ (1.25mmol/L) were mixed to form aqueous solution and used as a simulated exudate fluid (SEF). Firstly, the sample was weighed ($m_1$) and placed at the upper edges of modified Paddington cup. The modified Paddington cups (Figure 1) [test area of 4.00 $cm^2$ (A)] were filled with 20 mL of SEF prior to the placement of the samples. The cup (system) was then weighed ($m_2$), inverted (see Figure 1b) so that the dressing made contact with the SEF. The solution was kept in a 37°C environment with a relative humidity of under 20% for 24 hours ($t$) in a temperature and humidity-controlled incubator. Following the test, the cups were taken out of the incubator and left to equilibrate for 30 minutes at ambient temperature before being reweighed. Both sample ($m_3$) and system ($m_4$) were reweighed on the analytical balance. The following equations were used to get the swelling capacity, MVTR and FHC.

$$Swelling\ Capacity = \frac{m3-m1}{t\ .\ A} \quad (1)$$

$$MVTR = \frac{m4-m2}{t\ .\ A} \quad (2)$$

$$FHC = Swelling\ capacity + MVTR \quad (3)$$

(a) (b)

**Fig. 1.** Modified Paddington cup used in FHC analysis

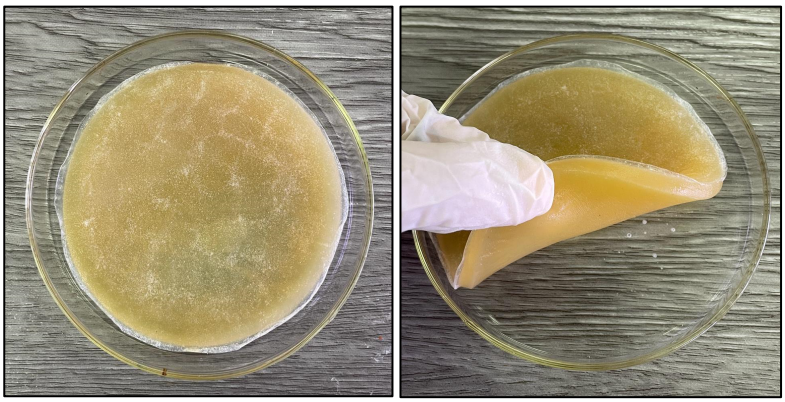
### *2.5 Microbial Penetration Test*

The Wittaya-areekul and Prahsarn method [21] was used to examine the membrane's resistance to microbial penetration. Glass vials containing 50 mL of TSB (tryptone soy broth) medium were filled with polyvinyl chloride (PVC) fittings that had been sterilised using ethanol 70% (v/v). After that, the glass vials and PVC fittings were placed together with the membrane samples (5 cm in diameter). All samples were cleaned with PBS after being decontaminated with ethanol 70% (v/v) for two hours. A sealed system covered in PVC film served as the negative control. The open vial served as the positive control. All systems were incubated for 7 days under room conditions. Any cloudiness on the TBS medium was attributed to microbial contamination.

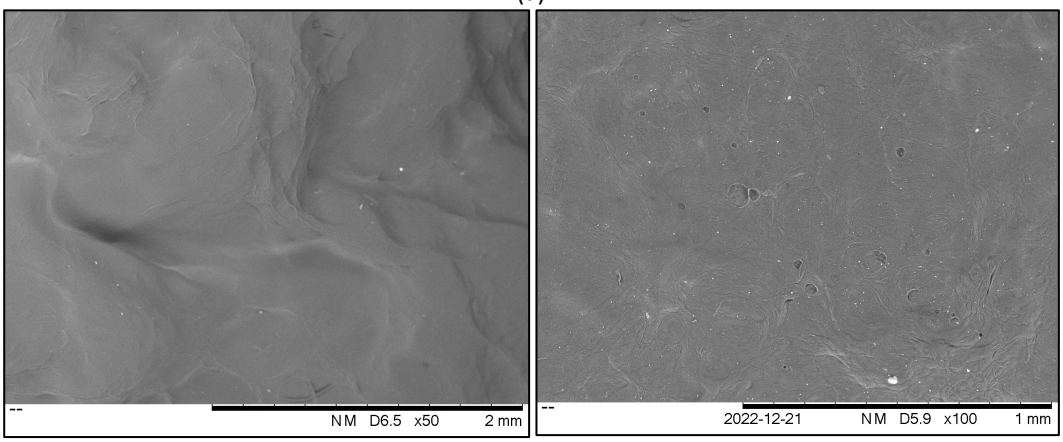
## 3. Results

### *3.1 Structure and Morphology*

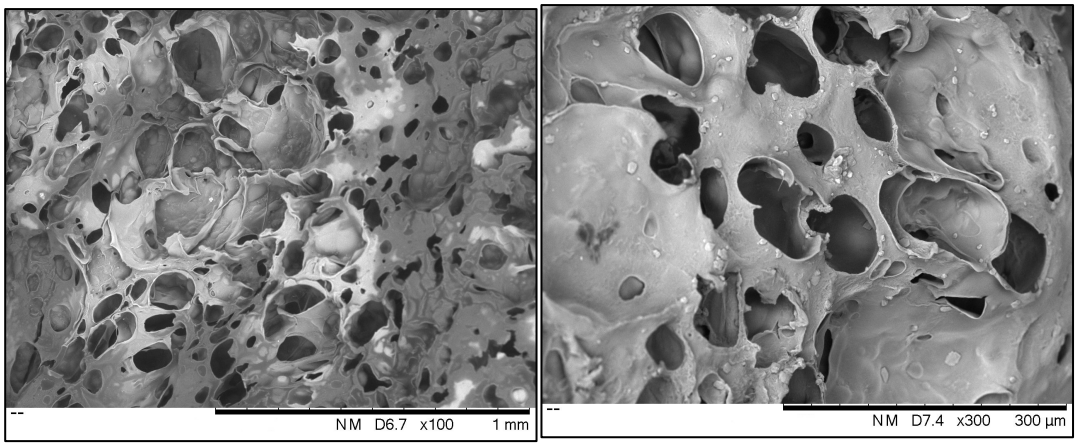
The image of the KGM asymmetric membrane is shown in Figure 2(a). The sample presented a hard, flat top layer, meanwhile the bottom layer was a soft, porous structure. These macroscopic findings concur with the SEM micrographs displayed in Figure 2(b)-1 (c). The top layer showed a flat, smooth surface [Figure 2(b)], whereas the bottom layer showed a porous structure with a mean pore size of 145 µm and a porosity of 54%.



(a)




(b)




(c)

**Fig. 2.** (a) An image of the KGM-VCO asymmetric membranes; (b) SEM image of the KGM asymmetric membrane's occlusive surface (top layer); (c) SEM images of the porous KGM asymmetric membrane surface (bottom layer)

The asymmetric structure shown in the SEM micrographs is a result of differences in the water availability between the bulk and the top layers of the KGM-VCO solution. During the casting process, the top layers of the KGM-VCO solution present a water loss rate higher than that of the bottom layers due to the temperature gradient along the bulk of the solution. In addition, the jellification in alkaline solution creates both new hydrogen bonds and hydrophobic interactions among the KGM chains, thereby inhibiting the diffusion of water molecules between the layers of the solution. Since the KGM-VCO solution was partially dried, during the freezing step, ice crystals grow isotopically in the bulk of the water-rich bottom layers, leading to the formation of a porous structure. On the other hand, the freezing of the KGM rich layers on the top of the solution produced an occlusive surface.

### *3.2 Thermal stability*

The thermal stability and behaviour of KGM-VCO asymmetric membrane were principally assessed using the thermogravimetric analysis (TGA) method. Figure 3 indicates that between 200 °C and 500 °C, and notably between 200 °C and 400 °C, KGM powder loses mass owing to decomposition. This temperature range is associated with saccharide ring breakdown and polymer chain disintegration. Pure virgin coconut oil, on the other hand, loses weight mostly between 200 and 400 ℃, while it is completely decomposed over 500 ℃. For the VCO/KGM asymmetric membrane, the TGA curves exhibit distinct behaviour above and below the temperature of oil breakdown. The loss of moisture was attributed to the weight loss of the membrane from 25 to 150 °C [19]. The oil appears to maintain the membrane below the temperature of oil degradation, but when the temperature is higher than the temperature of oil degradation, the oil appears to favour the opposite.

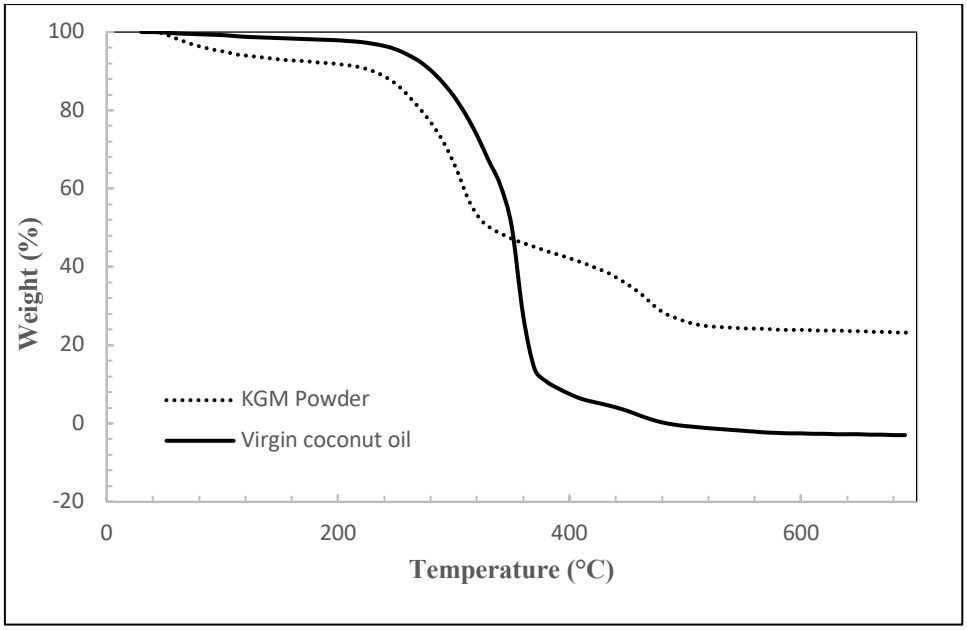



**Fig. 3.** Thermograms of virgin coconut oil (VCO) and KGM powder by using TGA methodology

Figure 3 revealed weight loss up to 300°C and 400°C as a result of these observations. This indicates that the inclusion of 0.1 percent VCO improves the thermal stability of the composite membrane owing to a degree of interaction between KGM and VCO, which is related to the findings of Ismail *et al.,* [22]. For 0.5% and 1.0% VCO, the oil appears to promote the reverse, resulting in a decrease in composite membrane thermal stability. The TGA results are consistent with prior investigations of KGM powders, demonstrating that the asymmetric membrane manufacturing technique had no effect on the thermostability of the pure polymer.

Figure 4 shows the thermogram of 3.5% (w/w) KGM asymmetric membrane with and without VCO. The result demonstrated that both membranes experienced weight loss at similar temperature range which was up to 50°C and 150 °C. However, KGM membrane with added VCO showed less weight loss as compared to pure KGM membrane. This indicates that the addition of VCO exhibits better outcome in increasing thermal stability of composite membrane due to certain degree of interaction between KGM and VCO. On the other hand, as compared to TGA curve of pure KGM (KGM powder), 0.5% of VCO oil appears to promote the reverse, resulting in a decrease in composite membrane thermal stability. Nevertheless, it still stables at temperature less than 100 °C and weight loss was just around 15%.

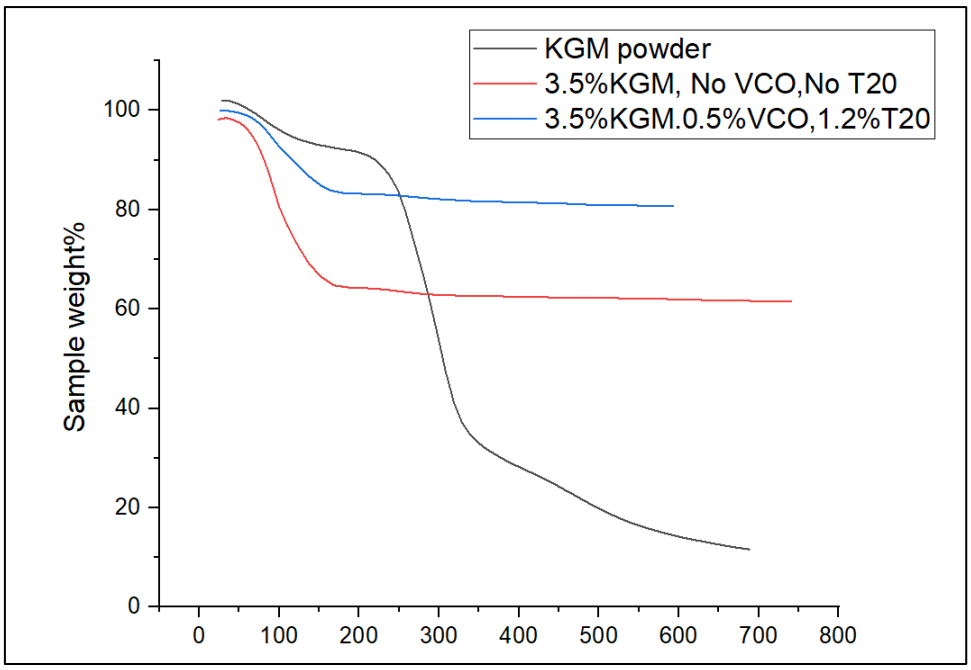



**Fig. 4.** TGA thermograms of 3.5% (w/w) KGM asymmetric membrane with and without virgin coconut oil

### *3.3 Fluid Handling Capacity (HFC) analysis*

A wound that is too dry may take longer or heal more slowly, but a wound with too much fluid may macerate or become infected. Applying a suitable dressing and removing it promptly to prevent maceration or adhesion creates the ideal healing environment. A healthy human skin has a moisture transmission rate of 204 g/m$^2$.24 h [23]. However, wounds to the skin may reduce its capacity to hold moisture. In order to avoid the maceration of healthy tissue and moisture loss from the wounded skin, wound dressings should also prevent exudate from building up in the injured area. The FHC test evaluates a wound dressing's ability to either absorb bodily fluids or maintain the optimal moisture levels for wound healing. Figure 5 displays the outcomes for swelling capacity, MVTR and FHC tests performed on the KGM-VCO membrane. The MVTR values of commercially available wound dressings with an asymmetric structure range from 1.67 to 12.35 g/10 cm$^2$/24 h [24]. As a result, the MVTR of 4.45 g/10 cm$^2$/24 h observed for our KGM-VCO membranes accords with the values discovered for reliable brands, such as Allevyn adhesive (Smith & Nephew, London, United Kingdom) and Active heal (Advanced Medical Solutions, Winsford, United Kingdom). Additionally, it is well-known that third-degree burns or wound lesions with granulation tissue often produce 3.4 to 5.1g of exudate per 10 cm$^2$ over a 24 hours period [3]. Therefore, it is possible that KGM-VCO membranes with FHC values of 5.6 g/10 cm$^2$/24 h could be employed to treat moderate to severe wound injuries with substantial exudate generation.

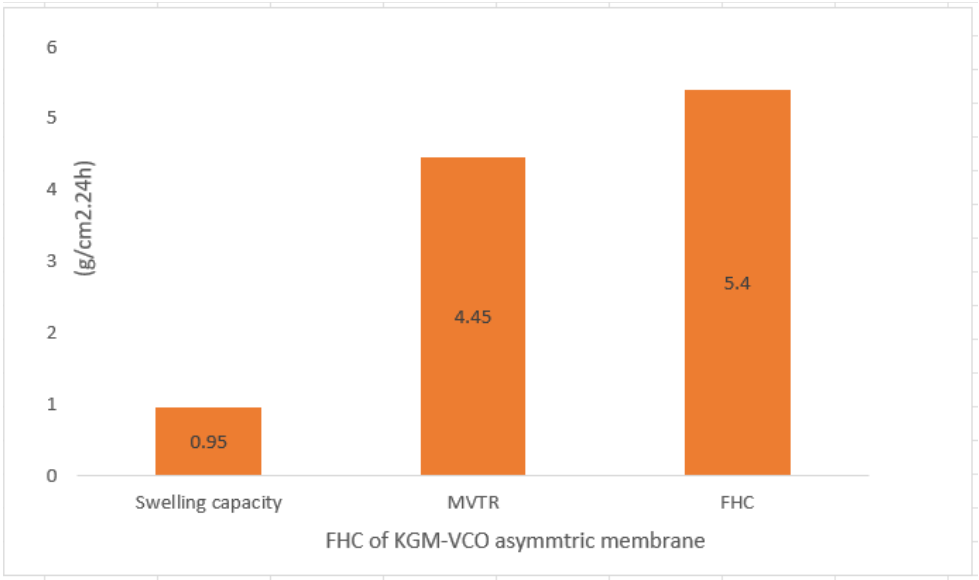



**Fig. 5.** Fluid Handling Capacity (HFC) of KGM-VCO asymmetric membrane

### *3.4 Microbial Penetration Test*

Physical and chemical protection against microbial intrusions is provided by human skin. Keratin-coated layers of epithelial cells serve as a strong physical barrier that prevents microbiological invasion. Furthermore, by eliminating any attached microorganisms, the regular renewal of skin cells may prevent microbial growth [19]. In terms of antibacterial control, wound dressings should function similarly to healthy human skin. Only the positive controls for the microbiological penetration tests showed cloudiness in the TBS medium. All the KGM-VCO membranes and negative controls, on the other hand, showed a transparent and uniform medium. These findings show that TSB constituted an ideal environment for microbial development and that no microbe was able to get through the KGM membranes as the top layer of sample has ability to prevent microbial penetration, like human skin.

## 4. Conclusions

Konjac glucomannan asymmetric membrane containing virgin coconut oil (VCO) were obtained with satisfactory properties for use as a dressing to heal wounds. The membranes obtained by the novel casting–freezing process presented an asymmetric structure composed of a flat film on the top layer and a porous structure on the bottom layer. The inclusion of VCO considerably changed the films' barrier characteristics while also enhancing their thermal stability. When compared to other commercially available wound dressings, these membranes displayed a satisfactory fluid handling capacity (HFC). Therefore, the asymmetric membrane of KGM-VCO is a promising material for wound dressing applications due to its moist environment, barrier properties and biocompatibility.

**Acknowledgement**
The authors are grateful to the financial support from University College TATI (UCTATI) for Short Term Grant (STG).

## References

[1] Boateng, Joshua S., Kerr H. Matthews, Howard NE Stevens, and Gillian M. Eccleston. "Wound healing dressings and drug delivery systems: a review." *Journal of pharmaceutical sciences* 97, no. 8 (2008): 2892-2923. https://doi.org/10.1002/jps.21210

[2] Xu, Rui, Hesheng Xia, Weifeng He, Zhichao Li, Jian Zhao, Bo Liu, Yuzhen Wang et al. "Controlled water vapor transmission rate promotes wound-healing via wound re-epithelialization and contraction enhancement." *Scientific reports* 6, no. 1 (2016): 24596. https://doi.org/10.1038/srep24596

[3] Morgado, Patrícia I., Ana Aguiar-Ricardo, and Ilídio J. Correia. "Asymmetric membranes as ideal wound dressings: An overview on production methods, structure, properties and performance relationship." *Journal of Membrane Science* 490 (2015): 139-151. https://doi.org/10.1016/j.memsci.2015.04.064

[4] Hinrichs, W. L. J., E. J. C. M. P. Lommen, Ch RH Wildevuur, and J. Feijen. "Fabrication and characterization of an asymmetric polyurethane membrane for use as a wound dressing." *Journal of applied biomaterials* 3, no. 4 (1992): 287-303. https://doi.org/10.1002/jab.770030408

[5] Chen, Yi, Lidan Yan, Tun Yuan, Qiyi Zhang, and Haojun Fan. "Asymmetric polyurethane membrane with in situ-generated nano-TiO2 as wound dressing." *Journal of Applied Polymer Science* 119, no. 3 (2011): 1532-1541. https://doi.org/10.1002/app.32813

[6] Mi, Fwu-Long, Yu-Bey Wu, Shin-Shing Shyu, Jen-Yu Schoung, Yaw-Bin Huang, Yi-Hung Tsai, and Jong-Yun Hao. "Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery." *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials* 59, no. 3 (2002): 438-449. https://doi.org/10.1002/jbm.1260

[7] Mi, Fwu-Long, Yu-Bey Wu, Shin-Shing Shyu, An-Chong Chao, Juin-Yih Lai, and Chia-Ching Su. "Asymmetric chitosan membranes prepared by dry/wet phase separation: a new type of wound dressing for controlled antibacterial release." *Journal of Membrane Science* 212, no. 1-2 (2003): 237-254. https://doi.org/10.1016/S0376-7388(02)00505-7

[8] Morgado, Patrícia I., Pedro F. Lisboa, Maximiano P. Ribeiro, Sónia P. Miguel, Pedro C. Simões, Ilídio J. Correia, and Ana Aguiar-Ricardo. "Poly (vinyl alcohol)/chitosan asymmetrical membranes: Highly controlled morphology toward the ideal wound dressing." *Journal of membrane science* 469 (2014): 262-271. https://doi.org/10.1016/j.memsci.2014.06.035

[9] Tang, Chuan, Yi-xin Guan, Shan-jing Yao, and Zi-qiang Zhu. "Preparation of drug-loaded asymmetric chitosan films towards wound dressing using supercritical solution impregnation." *Acta Polymerica Sinica* 6 (2014): 774-781. http://dx.doi.org/10.3724/SP.J.1105.2014.13363

[10] Xu, Hui, Jinming Chang, Yi Chen, Haojun Fan, and Bi Shi. "Asymmetric polyurethane membrane with inflammation-responsive antibacterial activity for potential wound dressing application." *Journal of Materials Science* 48 (2013): 6625-6639. https://doi.org/10.1007/s10853-013-7461-z

[11] Xie, Yu, Zeng-xing Yi, Jian-xun Wang, Tong-gang Hou, and Qiong Jiang. "Carboxymethyl konjac glucomannan-crosslinked chitosan sponges for wound dressing." *International journal of biological macromolecules* 112 (2018): 1225-1233. https://doi.org/10.1016/j.ijbiomac.2018.02.075

[12] Chambi, Hulda Noemi Mamani, and Carlos Raimundo Ferreira Grosso. "Mechanical and water vapor permeability properties of biodegradables films based on methylcellulose, glucomannan, pectin and gelatin." *Food Science and Technology* 31 (2011): 739-746. https://doi.org/10.1590/S0101-20612011000300029

[13] Shahbuddin, Munira, Anthony J. Bullock, Sheila MacNeil, and Stephen Rimmer. "Glucomannan-poly (N-vinyl pyrrolidinone) bicomponent hydrogels for wound healing." *Journal of Materials Chemistry B* 2, no. 6 (2014): 727-738. https://doi.org/10.1039/C3TB21640C

[14] Boateng, Laurene, Richard Ansong, William Owusu, and Matilda Steiner-Asiedu. "Coconut oil and palm oil's role in nutrition, health and national development: A review." *Ghana medical journal* 50, no. 3 (2016): 189-196. https://doi.org/10.4314/gmj.v50i3.11

[15] Nevin, K. G., and T. Rajamohan. "Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats." *Skin pharmacology and physiology* 23, no. 6 (2010): 290-297. https://doi.org/10.1159/000313516

[16] Zakaria, Z. A., M. N. Somchit, A. M. Mat Jais, L. K. Teh, M. Z. Salleh, and K. Long. "In vivo antinociceptive and anti-inflammatory activities of dried and fermented processed virgin coconut oil." *Medical Principles and Practice* 20, no. 3 (2011): 231-236. https://doi.org/10.1159/000323756

[17] Intahphuak, S., P. Khonsung, and A. Panthong. "Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil." *Pharmaceutical biology* 48, no. 2 (2010): 151-157. https://doi.org/10.3109/13880200903062614

[18] Shilling, Michael, Laurie Matt, Evelyn Rubin, Mark Paul Visitacion, Nairmeen A. Haller, Scott F. Grey, and Christopher J. Woolverton. "Antimicrobial effects of virgin coconut oil and its medium-chain fatty acids on Clostridium difficile." *Journal of medicinal food* 16, no. 12 (2013): 1079-1085. https://doi.org/10.1089/jmf.2012.0303

[19] Genevro, Giovana Maria, Reginaldo Jose Gomes Neto, Letícia de Almeida Paulo, Patrícia Santos Lopes, Mariana Agostini de Moraes, and Marisa Masumi Beppu. "Glucomannan asymmetric membranes for wound dressing." *Journal of Materials Research* 34, no. 4 (2019): 481-489. https://doi.org/10.1557/jmr.2018.463

[20] B. S. EN: 13726-1:2002, Test methods for primary wound dressings: Moisture Vapor Transmission Rate Permeable Film Dressings (2002).

[21] Wittaya-areekul, Sakchai, and Chureerat Prahsarn. "Development and in vitro evaluation of chitosan–polysaccharides composite wound dressings." *International journal of pharmaceutics* 313, no. 1-2 (2006): 123-128. https://doi.org/10.1016/j.ijpharm.2006.01.027

[22] Ismail, Nur Arifah, Siti Fatimah Mohamad, Maizatul Akma Ibrahim, and Khairul Anuar Mat Amin. "Evaluation of gellan gum film containing virgin coconut oil for transparent dressing materials." *Advances in Biomaterials* 2014 (2014). https://doi.org/10.1155/2014/351248

[23] Yannas, I. V., J. F. Burke, M. Warpehoski, P. Stasikelis, E. M. Skrabut, D. P. Orgill, and D. Giard. "Design principles and preliminary clinical performance of an artificial skin." 1982. https://doi.org/10.1021/ba-1982-0199.ch029

[24] Thomas, S and Young S. Exudate-handling mechanisms of two foam-film dressings. Journal of Wound Care, 17 (2008): 309. https://doi.org/10.12968/jowc.2008.17.7.30524