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Fabrication of Natural and Bio-Based Wound Healing Material from Alginate and Beeswax

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Abstract. Alginate is a natural polysaccharide derived from brown algae and exhibits the following properties: biocompatible, biodegradable and non-toxic. Beeswax is a secretion that is produced by young working bees. For this research project, alginate and beeswax are homogenized together to form bio-based films for wound healing applications. Solution casting method was used to fabricate the films of 1 wt% and 2 wt% sodium alginate (SA) with 0-5 g beeswax. The films were then characterized and tested using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Atomic Force Microscopy (AFM), and contact angle measurement. FTIR spectra revealed that all films showed spectra from both SA and beeswax pellet, which implies that alginate and beeswax coexist in the film. SEMimages showed smooth surfaces for films of 1 wt% and 2 wt% SA with 0 g beeswax and rough surfaces with beeswax particles for films of 1 wt% and 2 wt% SA with 1-5 g beeswax. AFM results revealed that the surface roughness of alginateand beeswax films increased with an increasing amount of beeswax and film of 2 wt% SA with 5 g beeswax recorded the highest surface roughness value. Contact angle measurement revealed that as more beeswax was introduced into alginate solution, the contact angle value increases due to the hydrophobic nature of beeswax. It can be concluded that these alginate and beeswax films can be applied as both hydrophilic and hydrophobic wound dressing materials.

INTRODUCTION

Skin is the largest organ of a human body. It serves the following important functions: natural barrier to protect the internal organs from the outer environment, prevent pathogens from entering the human body and keep the body from dehydration. However, skin can get injured very easily. Once injured, its function will be compromised, making it easier for microorganisms to enter the human body and form colonies that will infect the damaged skin area. An infected wound takes a longer time to heal and in worst case scenario, it can also lead to life threatening complications [1]. Hence, wound dressings were developed and have become the top-priority in wound treatment. An ideal wound dressing should demonstrate the following characteristics: good moisture control, biocompatible, biodegradable, non-toxic and wound soothing [2]. These characteristics are crucial in the development of wound dressings for an effective and a successful wound healing. Presently, synthetic-based and natural-based wound dressings are widely available in market. Natural-based wound dressings are more favored due to their significant attribute such as biocompatibility, biodegradability and most importantly, similarity with human tissue in terms of structure [3]. Apart from that, they also showed excellent results in modeling disrupted tissues, followed by skin and tissue renewal [4]. Alginate, collagen and chitosan are some of the most widely used natural biopolymers in fabricating natural-based wound dressings [3].

Alginate is a natural polysaccharide made up of two saccharide epimers: β-(1-4)-D-mannuronic (M unit) and α-L-guluronic acid (G unit). These units are linked together via glycosidic linkage between C1and C4 [5]. Alginate is extracted from the cell walls of brown algae and it demonstrates the following attributes: non-toxic, biocompatible, biodegradable and good hydrophilicity. Hydrophilicity is a crucial characteristic when designing a wound dressing as wound requires a continuous wet surrounding that would assist hemostasis process, ensuring a successful and effective wound healing [6]. However, hydrophilic wound dressings are not suitable for all types of wound. Hence, hydrophobic

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wound dressings are developed to cater condition where cell proliferation around the wounded area is not favored, for example, tendon injury [7].

Beeswax is secretion that comes from young working bees. It is made up of a combination of 300 elements such as hydrocarbons, fatty acids, free fatty alcohols, esters and foreign matters such as propolis, pollen, fragments of floral elements and pollution [8]. These combinations are affected by the genetics of the bees and their diet [9]. Beeswax has long been used in pharmaceutical and traditional medicine since ancient Egypt. It was used to treat skin injuries such as wounds and burns [8]. Beeswax is hydrophobic and hence for this project, it was combined with alginate to make it suitable for other types of wounds [7]. Films of alginate and beeswax were prepared, characterized and evaluated for hydrophilicity and hydrophobicity properties.

METHODOLOGY

Materials

For this research project, sodium alginate (SA) and bleached beeswax were used as raw materials, in the form of powder and pellet, respectively. Both raw materials were purchased from Sigma-Aldrich (CAS number (SA): 9005-38-3 and CAS number (bleached beeswax): 8012-89-3).

Fabrication of Alginate and Beeswax Films

Alginate and beeswax films were fabricated via solution casting method. 1 wt% and 2 wt% SA solution with 0-5 g beeswax were prepared by diluting in distilled water. The mixture was stirred on a hot plate with magnetic stirrer for 45 minutes to achieve a homogeneous mixture. Then, the mixture was removed from the hot plate and left for 24 hours to allow the mixture to stabilize. After that, 10 mL of each solution was poured into a petri dish. Then, the mixture was dried at room temperature for 5 to 6 days to form a solid film.

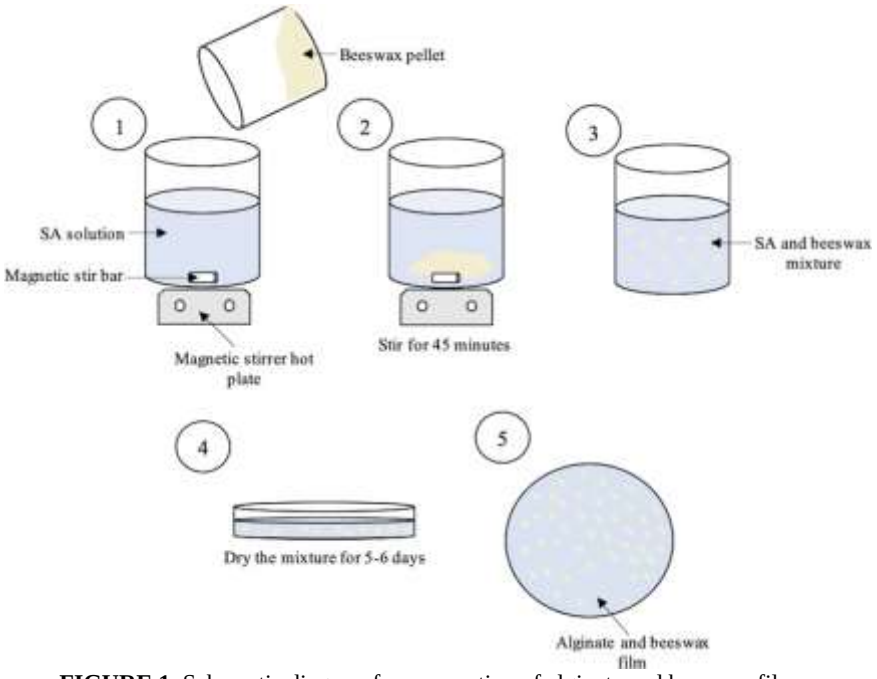

FIGURE 1. Schematic diagram for preparation of alginate and beeswax films.

Characterization Of Alginate and Beeswax Films

Alginate and beeswax films were cut into small squares measuring approximately 1 cm × 1 cm for characterization purposes. The functional groups of the films were characterized by FTIR using PerkinElmer Spectrum 100 FT-IR

Spectrometer (PerkinElmer Inc., USA). The films were analyzed directly. The wavenumber range was from 4000 to 600 cm^{-1} at the resolution of 4 cm^{-1} with scanning number of 32. The surface of alginate and beeswax films were visualized using Hitachi VP-SEM SU1510 (Hitachi Ltd., Japan). The films were fixed on stub by double sided carbon tape, sputtered with a thin layer of gold and assembled onto the machine for analysis. The magnification used was 500x magnification for all sample. The working voltage was 15 kv. The surface topography of alginate and beeswax films were determined using Park XE-100 AFM (Park Systems Corp, Korea). Films were taped on glass slides before being fixed onto the sample disk inside the machine. The surface topography was then analyzed. The contact angle for alginate and beeswax films were measured using VCA Optima (AST Products, USA). Films were taped on glass slides. The glass slides were then fixed onto the stage of the machine. Water droplet was then dropped onto the surface of the film. A camera captures the image and the contact angle value was measured.

RESULTS AND DISCUSSION

FTIR Spectra of Alginate and Beeswax Films

The functional group of alginate and beeswax films were analyzed using FTIR. Figure 2 shows the FTIR spectra for films of 1 wt% and 2 wt% SA. These spectra were used as a reference sample against spectra of alginate and beeswax (1-5 g) films. For films of 1 wt% and 2 wt% SA, broad bands at 3263 cm^{-1} and 3256 cm^{-1} were detected, respectively, contributed by OH stretching band. Small, medium bands were observed at 2936-2937 cm^{-1} in both films, contributed by the presence of -CH stretching vibration band. Stretching bands at 1592 cm^{-1}, 1594 cm^{-1} and 1409 cm^{-1} were due to the existence of carboxylic group as the backbone of SA [10]. The observed band at 1084 cm^{-1} in both films shows the existence of C-C and C-O stretching bands. For films of 1 wt% and 2 wt% SA, the band that recorded the highest intensity was at 1025 cm^{-1} and 1026 cm^{-1}, respectively, which might be due to strong OH binding vibration. Bands observed at 949 cm^{-1}, 811 cm^{-1} and 812 cm^{-1} were contributed by guluronic and mannuronic acids, confirming the existence of G-blocks and M-blocks in SA [10].

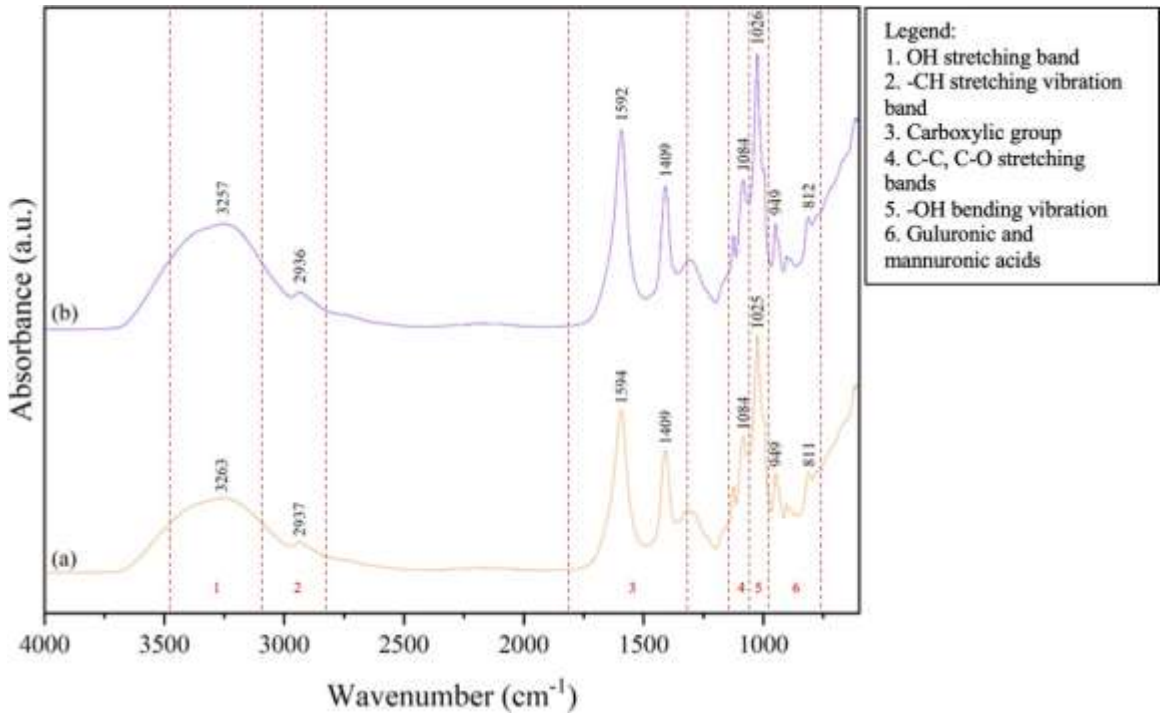

FIGURE 2. FTIR spectra for films of (a) 1 wt% SA with 0 g beeswax and (b) 2 wt% SA with 0 g beeswax.

Figure 3 shows the FTIR spectrum for beeswax pellet. Peaks observed at 2921 cm^{-1}, 2849 cm^{-1}, 1463 cm^{-1} and 720 cm^{-1} belong to hydrocarbon absorption bands, contributed by CH_2 asymmetric stretching vibration, CH_2 symmetric stretching vibration, CH_2 scissor deformation and CH_2 rocking mode, respectively. Bands observed at 1736 cm^{-1}

indicates the presence of esters and free fatty acids, which was contributed by C=O stretching vibration. The band observed at 1712 cm^{-1} were due to the existence of ester and free fatty acids. At 1171 cm^{-1}, the band exists due to C=O stretching and C-H bending vibrations, indicating the presence of esters. These findings are almost similar to a study carried out on virgin beeswax but with a slight shift in the wavenumber [11].

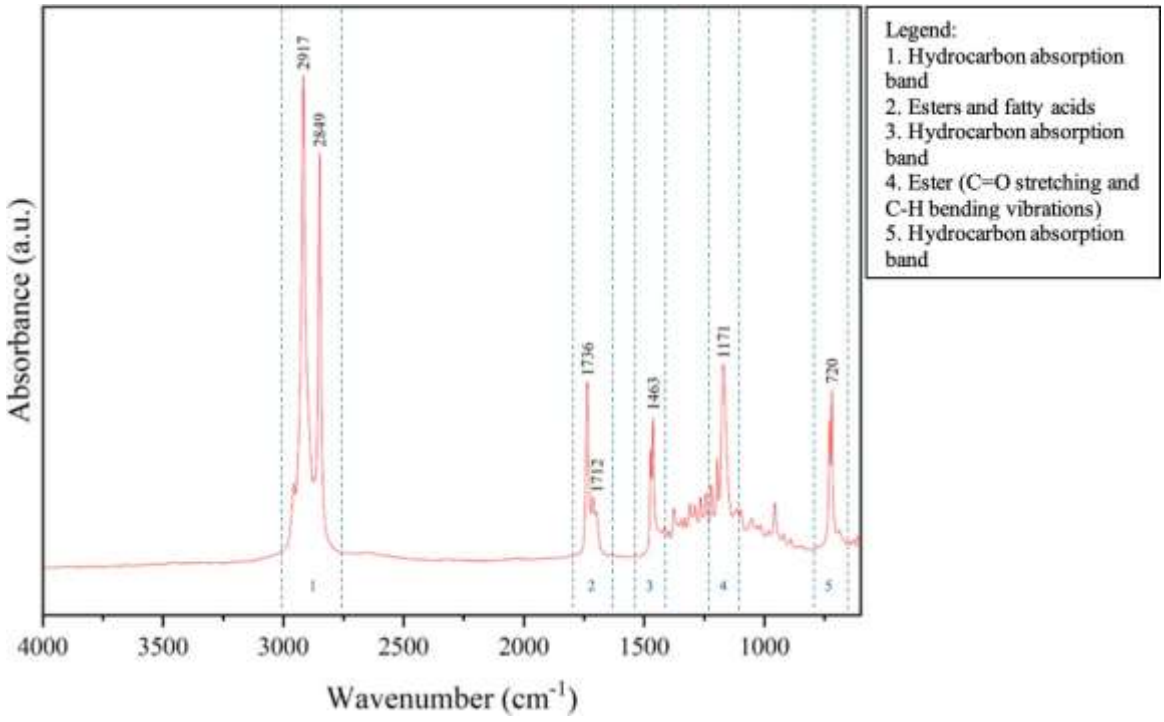

FIGURE 3. FTIR spectrum for beeswax pellet.

Figure 4 and Figure 5 show the FTIR spectra for films of 1 wt% SA with 1-5 g beeswax and films of 2 wt% SA with 1-5 g beeswax, respectively. Overall, films of 1 wt% and 2 wt% SA with 1-5 g beeswax recorded both absorption bands from SA and beeswax pellet, with different intensities and slight shift in wavenumber. For films of 1 wt% SA, films with 1 g and 5 g beeswax showed the highest intensities, followed by film with 3 g beeswax with moderate intensity and films of 2 g and 4 g with the lowest intensities. For films of 2 wt% SA, the intensities are generally lower than that of 1 wt% SA. It was observed that the intensities at certain wavenumber showed a constant increase with increasing amount of beeswax. This might be due to increase in amount of the functional group of beeswax (per unit volume) which associated with the molecular bond.

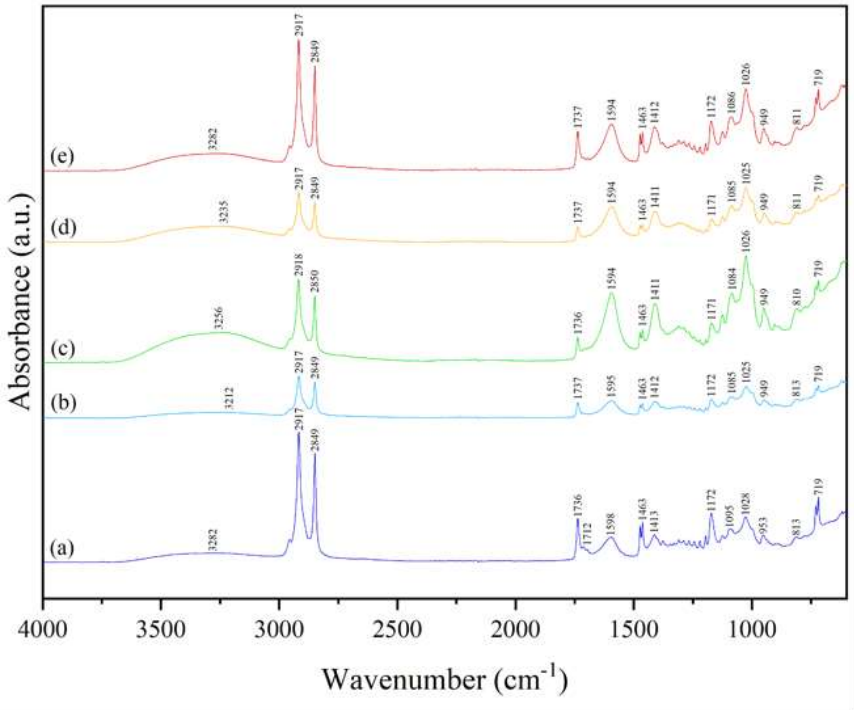

FIGURE 4. FTIR spectra for films of 1 wt% SA with (a) 1 g beeswax, (b) 2 g beeswax, (c) 3 g beeswax, (d) 4 g beeswax and (d) 5 g beeswax.

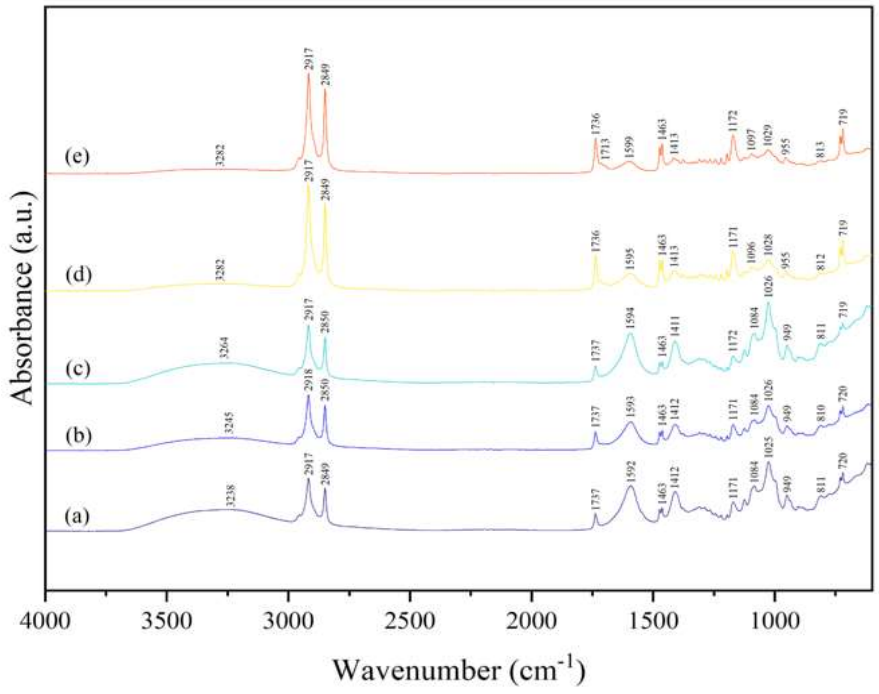

FIGURE 5. FTIR spectra for films of 2 wt% SA with (a) 1 g beeswax, (b) 2 g beeswax, (c) 3 g beeswax, (d) 4 g beeswax and (e) 5 g beeswax.

Surface Morphology for Alginate and Beeswax Films

Alginate and beeswax films were observed under SEM for surface morphology. Figure 6 and Figure 7 show the surface morphology for films of 1 wt% and 2 wt% SA with 0-5 g beeswax. Films of 1 wt% and 2 wt% SA with 0 g beeswax showed smooth surfaces without small particles, indicating that SA powder dissolved fully during stirring process. Films of 1 wt% and 2 wt% SA with 1-5 g beeswax demonstrate surfaces with small particles, indicating the presence of beeswax. Film of 1 wt% SA showed more presence of beeswax particle compared to film of 1 wt% SA with 2 g beeswax, when theoretically, the latter should be the one exhibiting more beeswax particles. This might be due to uneven particle distribution on the film surface. The particle size varies from small to large.

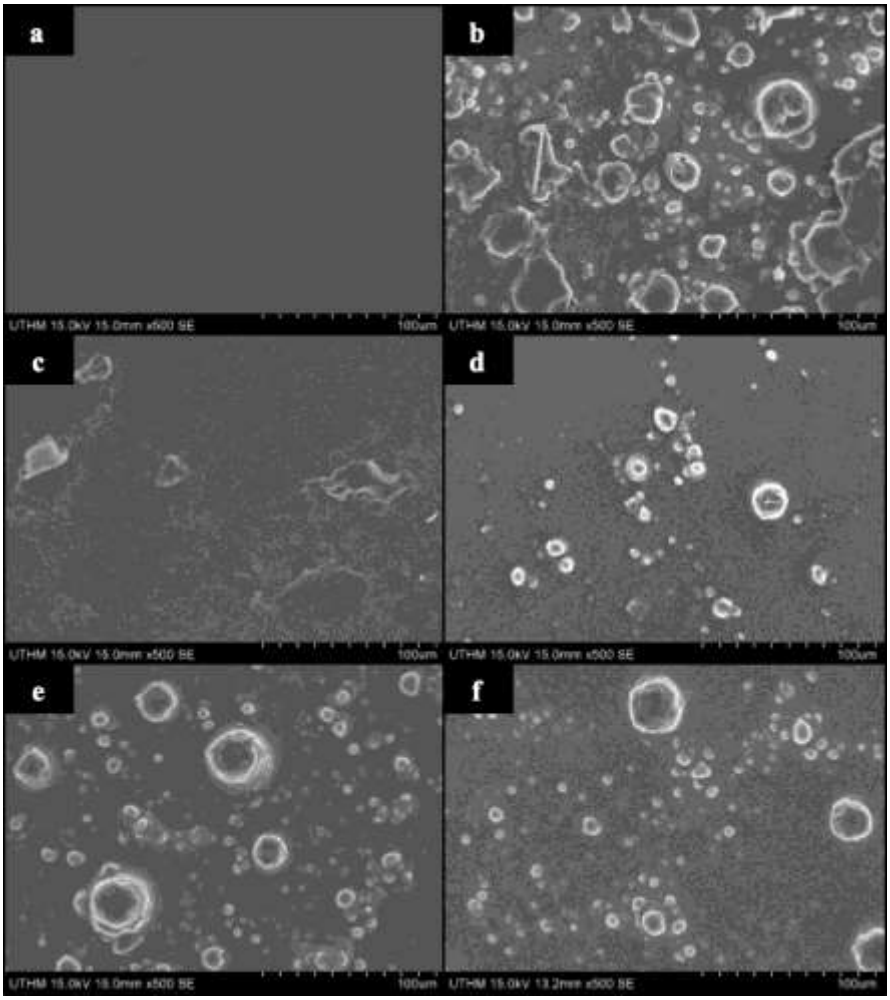

FIGURE 6. SEM images of films of 1 wt% SA with (a) 0 g beeswax, (b) 1 g beeswax, (c) 2 g beeswax, (d) 3 g beeswax, (e) 4 g beeswax and (f) 5 g beeswax (brighter areas represent beeswax particles).

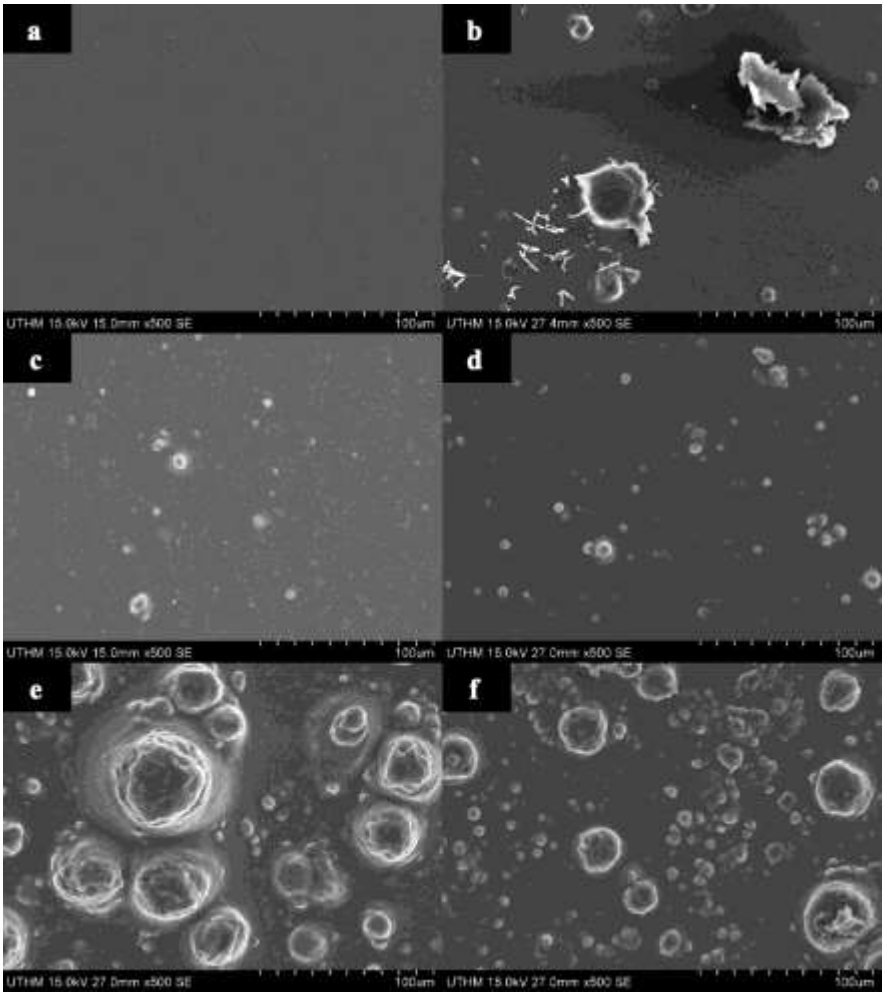

FIGURE 7. SEM images of films of 2 wt% SA with (a) 0 g beeswax, (b) 1 g beeswax, (c) 2 g beeswax, (d) 3 g beeswax, (e) 4 g beeswax and (f) 5 g beeswax (brighter areas represent beeswax particles).

Surface Roughness for Alginate and Beeswax Films

The surface topography for alginate and beeswax films were demonstrated via AFM, as depicted in Figure 8 and Figure 9. For films of 1 wt% SA with 0-5 g beeswax, it was observed that the structure of the aggregates is almost uniform on all films, with some films having bigger and more prominent aggregate structure. Film of 1 wt% SA with 4 g beeswax demonstrate a more prominent aggregate and larger structure, relative to its highest surface roughness value among all sample. Film of 1 wt% SA with 5 g beeswax showed the second highest surface roughness value, with slightly lower aggregate structure heights compared to that of 1 wt% SA with 4 g beeswax. For films of 2 wt% SA with 0-5 g beeswax, alginate film with 0 g beeswax show the lowest surface roughness value, consistent with it having lower aggregate structure height. Film of 2 wt% SA with 5 g beeswax demonstrate the highest surface roughness value, relative to its bigger and higher aggregate structure. Overall, the films showed an increase in surface roughness value with increasing amount of beeswax.

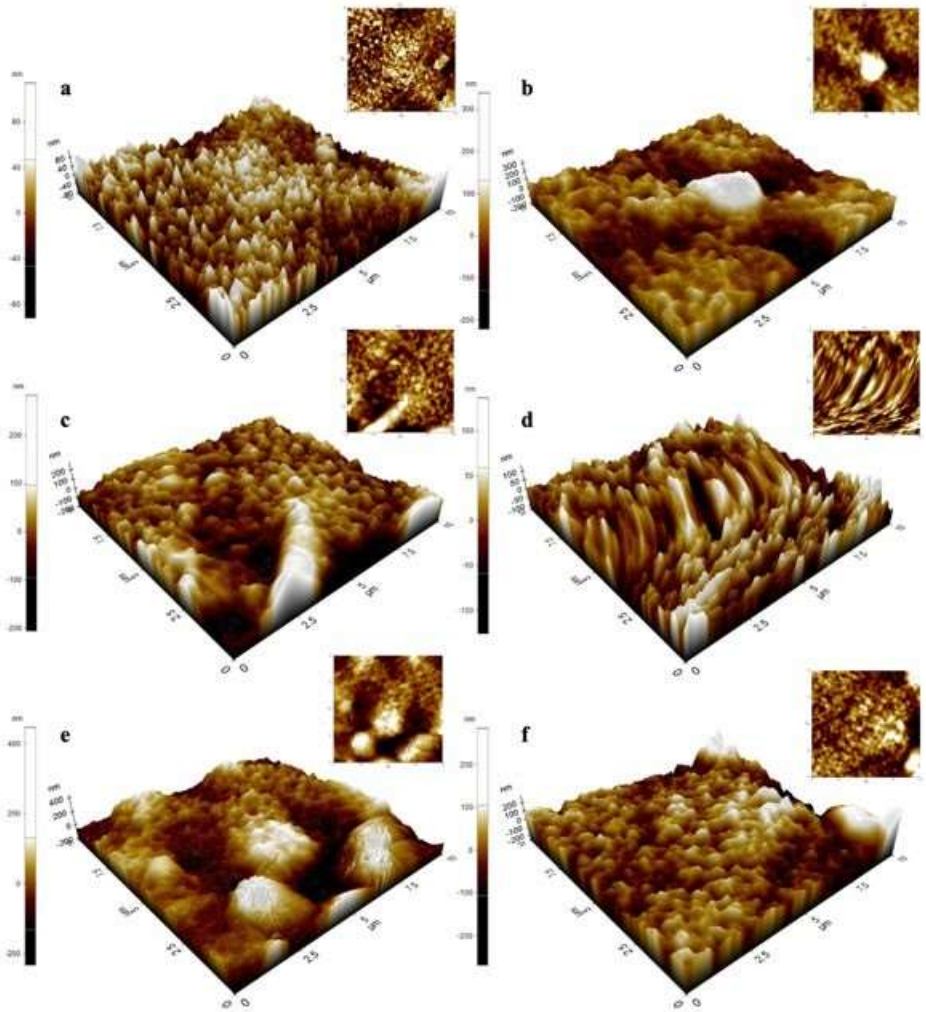

FIGURE 8. AFM images of films of 1 wt% SA with (a) 0 g beeswax, (b) 1 g beeswax, (c) 2 g beeswax, (d) 3 g beeswax, (e) 4 g beeswax and (f) 5 g beeswax.

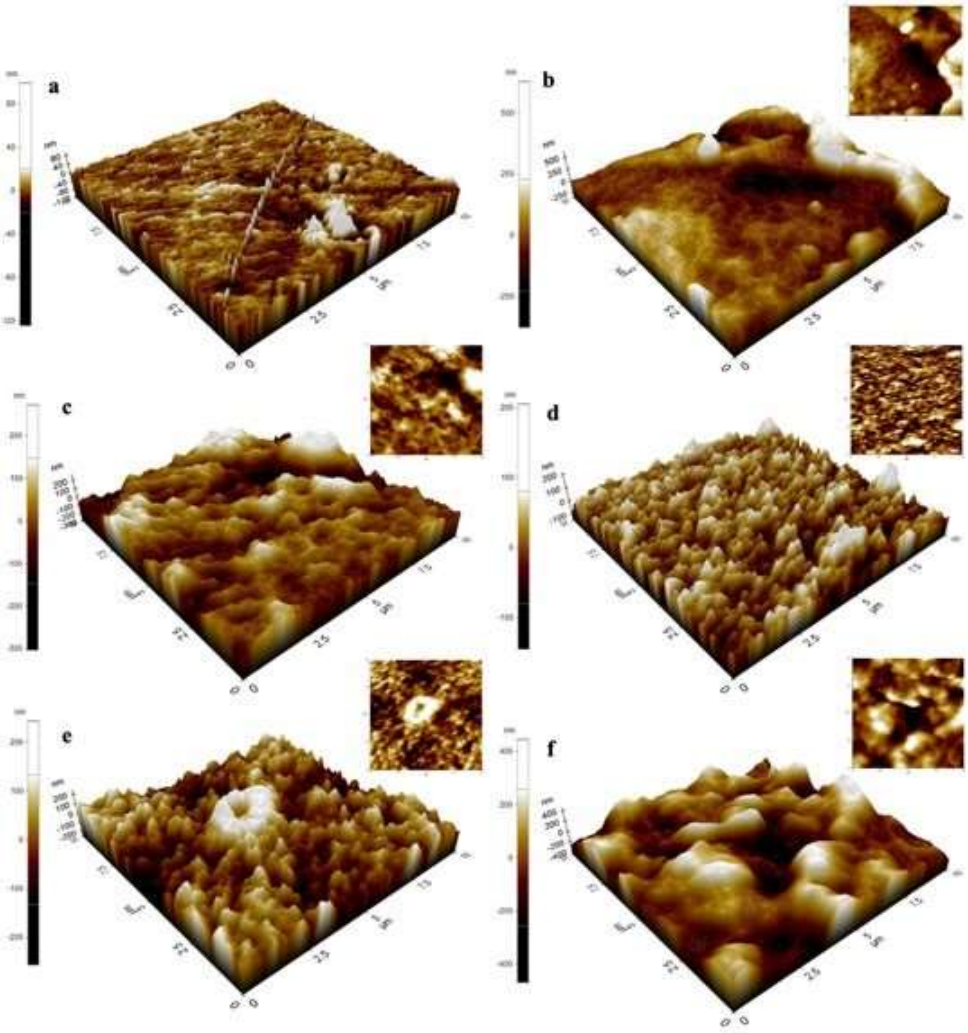

FIGURE 9. AFM images of films of 2 wt% SA with (a) 0 g beeswax, (b) 1 g beeswax, (c) 2 g beeswax, (d) 3 g beeswax, (e) 4 g beeswax and (f) 5 g beeswax.

Contact Angle Measurement Result

The hydrophilicity and hydrophobicity of the films were measured via contact angle measurement and the contact angle values were illustrated in Figure 10. For films of 1 wt% SA with 0-5 g beeswax, the contact angle values recorded an increase with increasing amount of beeswax, relative to the hydrophobic nature of beeswax which repels water. Film of 1 wt% SA with 0 g beeswax show the lowest contact angle value ($58.07 \pm 10.52°$), related to the absence of beeswax. The contact angle values recorded an increment for films of 1 wt% SA with 1 g and 2 g beeswax before decreasing form film containing 3 g beeswax. The contact angle values then recorded an increase for films of 1 wt% SA with 4 g and 5 g beeswax, with the latter showing the highest contact angle value ($91.40 \pm 8.95°$), relative to its highest beeswax content. For films of 2 wt% SA with 0-5 g beeswax, it was observed that the values for contact angle increases consistently with increasing amount of beeswax. Film of 2 wt% SA with 5 g beeswax recorded the highest reading (98.50 ± 5.26), consistent with its high beeswax content. Theoretically, surfaces with contact angle value of less than 90° are deemed as hydrophilic surface and vice versa. Overall, films of 1 wt% SA with 0-4 g beeswax and 2 wt% SA with 0-3 g beeswax demonstrate hydrophilic surfaces since the contact angle values are less than 90° while films of 1 wt% SA with 5 g beeswax and 2 wt% SA with 4-5 g beeswax exhibit hydrophobic surfaces since the contact angle values exceed 90°.

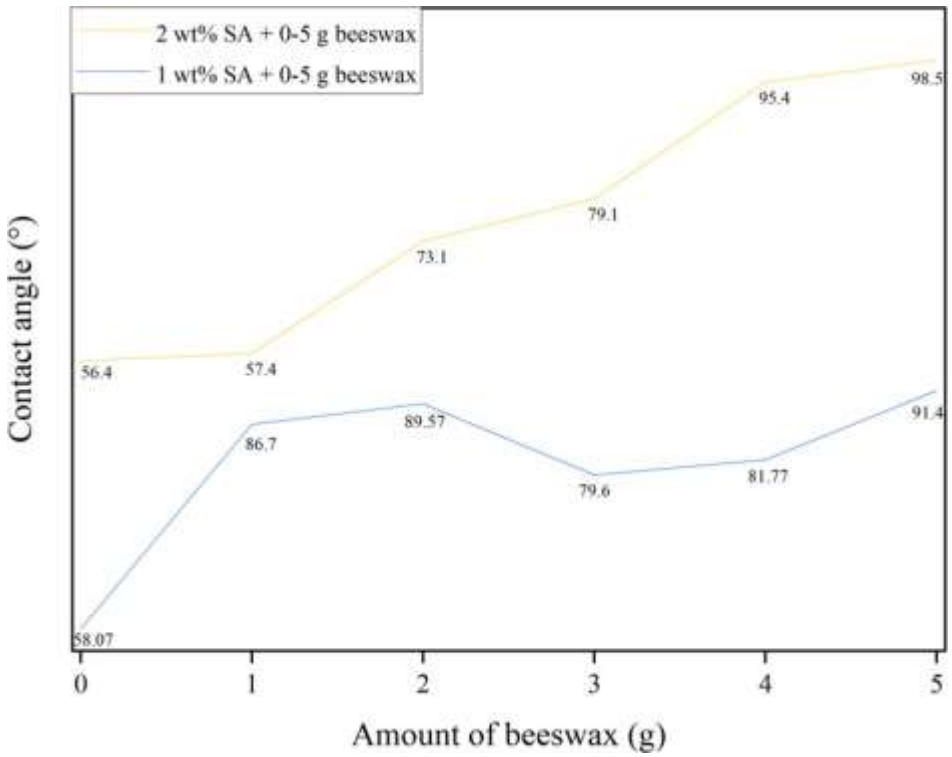

FIGURE 10. Contact angle values for films of 1 wt% and 2 wt% SA with 0-5 g beeswax.

CONCLUSION

Films of 1 wt% and 2 wt% SA with 0-5 g beeswax were successfully fabricated via solution casting method. Characterization and testing such as FTIR, SEM, AFM and contact angle measurement were carried out on all films. Overall, films of 1 wt% and 2 wt% SA with 0-5 g beeswax demonstrated vibration bands from SA and beeswax pellet. The SEM images presented smooth surfaces for alginate films with 0 g beeswax and surfaces with particles for alginate films with 1-5 g beeswax. As more beeswax was added into the solution, more particles were observed on the film surface. AFM results reveal a consistent increase in surface roughness with increasing amount of beeswax. Contact angle values revealed that as more beeswax was added, the film surface became more hydrophobic, relative to the hydrophobic nature of beeswax. Based on these findings, it can be concluded that alginate and beeswax films can act as both hydrophilic and hydrophobic dressings. On the other hand, further study must be carried out to study the antibacterial properties of the wound dressings.

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REFERENCES

1. R. Deepachitra, R. Pujitha Lakshmi, K. Sivaranjani, J. Helen Chandra, and T. P. Sastry, J. Chem. Pharm. Sci.**8**, 324–329 (2015).
2. E. Rezvani Ghomi, S. Khalili, S. Nouri Khorasani, R. Esmaeely Neisiany, and S. Ramakrishna, J. Appl. Polym. Sci.**136**, 47738 (2019).
3. G. D. Mogoşanu and A. M. Grumezescu, Int. J. Pharm.**463**, 127–136 (2014).

4. E. A. Kamoun, E. R. S. Kenawy, and X. Chen, J. Adv. Res. **8**, 217–233 (2017).
5. R. J. Coleman, G. Lawrie, L. K. Lambert, M. Whittaker, K. S. Jack, and L. Grndahl, Biomacromolecules **12**, 889–897 (2011).
6. R. Ahmad Raus, W. M. F. Wan Nawawi, and R. R. Nasaruddin, Asian J. Pharm. Sci **16,** 280-306 (2021).
7. J. Zou *et al.*, Mater. Sci. Eng. C. **116**, 111166 (2020).
8. F. Fratini, G. Cilia, B. Turchi, and A. Felicioli, Asian Pac. J. Trop. Med. **9**, 839–843 (2016).
9. R. Buchwald, M. D. Breed, A. R. Greenberg, and G. Otis, J. Exp. Biol. **209**, 3984–3989 (2006).
10. C. R. Badita, D. Aranghel, C. Burducea, and P. Mereuta, Rom. J. Phys. **65**, 1–8 (2020).
11. L. Svečnjak, G. Baranović, M. Vinceković, S. Prđun, D. Bubalo, and I. T. Gajger, J. Apic. Sci. **59**, 37–49 (2015).