Research Progress in Mechanical and Manufacturing Engineering Vol. 3 No. 1 (2022) 929-937

RPMME

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/rpmme
e-ISSN : 2773-4765

The Fabrication of Alginate and Curcumin Biocomposite Film for Wound Healing Applications

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DOI: https://doi.org/10.30880/rpmme.2022.03.01.099
Received 01 Dec 2021; Accepted 01 April 2022; Available online 30 July 2022

Abstract: Wound healing is an arduous and dynamic process that regenerates the debilitated tissue integrity. In this study, a biocomposite film of alginate and curcumin was fabricated using the solution casting method. The physicochemical and antibacterial properties of the films were investigated. The films were fabricated with 0.2%, 0.4%, 0.6%, 0.8% and 1.0% curcumin concentrations and they were characterised using the digital micrometer, Fourier transform infrared spectroscopy (FTIR), goniometer and atomic force microscope (AFM), and the antibacterial assay was done using the disc diffusion method. The values of the thickness and the surface roughness from AFM findings increased with the increased concentration of curcumin. It was found that the successful intermolecular interactions between curcumin and alginate were also visible in the film's FTIR spectrum. Next, the contact angle measurement also exhibits a similar pattern when the value is increased with the concentrations of curcumin as well. The antibacterial study on biocomposite film with *S.aureus* was evaluated. Unfortunately, it was found there is still no resistance to the bacterial growth in the samples. This may be due to the low concentration of curcumin applied or used in this study. Nevertheless, this biocomposite film could possibly be developed as a wound dressing with further improvements.

Keywords: Alginate, Curcumin, Antibacterial, FTIR, Surface Roughness, Contact Angle

1. Introduction

Wounds are usually categorised based on the severity of the injuries, location, aetiology, their depth, and clinical appearance (Clinical Effectiveness Committee, 2019). The assessment is vital to ensure appropriate wound management for the wound healing process. Wound healing is an arduous and dynamic process that regenerates the debilitated tissue integrity. Skin wound healing is a crucial procedure for survival, finalising wound closure due to skin's significant role as the body barrier that protects from physical impacts, chemicals, radiation, and microorganisms. The treatments for chronic wounds are time-consuming and costly, which has promoted the development of new dressing materials. This development includes the usage of polymers and bioactive compounds from medicinal

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plants that will act as therapeutic agents due to their versatile properties, biocompatibility, and their abundant source [2].

Alginate is a naturally occurring multifunctional polymer which can be derived from brown seaweed (*Phaeophyceae*). The alginate structure is mainly composed of (1-4)-linked β-D-mannuronic acid (M units) and α-L-guluronic acid (G units) monomers, and these monomers strongly influence the characteristics of the alginate such as its viscosity, water uptake ability, and gelation. Alginate is commonly used for wound dressing in wound healing applications because it can remove excess wound fluid, reducing bacterial infections in the affected area, and physiologically preserving the moist environment to optimally facilitate wound healing [3]. Commonly, the alginate-based wound dressings that are often used on serious wounds are produced via the ionic cross-linking of an alginate solution and calcium ions from calcium chloride ($CaCl_2$) because crosslinked alginate shows better mechanical strength, water barrier properties, cohesiveness, and rigidity. According to Szekalska et al. (2016), alginate was shown to have a bacteriostatic effect against a wide range of bacteria species, including *Pseudomonas, Escherichia, Proteus, and Acinetobacter* [4]. A few mechanisms have been documented to clarify the antimicrobial activity of alginate. The interaction of the negatively charged alginate with the outer bacterial cellular surface has caused the intracellular substances' disruption and leakage [5] [6].

Curcumin is a bioactive compound derived from Curcuma longa, also famously known as turmeric. Since ancient times, curcumin has been used to treat various diseases due to its potency, affordability, and rich antioxidant source. Due to this property, it is poorly soluble in neutral solvents like water, but it is highly soluble in organic solvents like ethanol, isopropanol, and acetone [7]. Curcumin's degradation is pH-dependent, and the degradation will be more rapid at or higher physiological pH, which will cause major drawbacks in its therapeutic applications. Curcumin was also found to promote skin wound healing by its involvement in the proliferative phase that includes the formation of tissue granulation and collagen build-up, improving fibroblast proliferation and vascular density, epithelial regeneration, and apoptosis of unnecessary cells. Mohanty *et al.* (2012) provided evidence that myofibroblast was abundant as well as the increased granulation formation tissue that resulted in rapid re-epithelization with the presence of curcumin for diabetic rats' wound treatment [8]. Curcumin has been shown to inhibit the development of two important cytokines, tumour necrosis factor-alpha (TNF-) and interleukin-1 (IL-1) that are released from monocytes and macrophages that play a crucial role in regulating inflammatory responses [9]. Curcumin has been proven to have therapeutic potential due to its versatile properties, thus making it a great choice to be used in wound healing applications.

In this study, an alginate-based antibacterial biocomposite film incorporating curcumin will be fabricated for wound healing application. The film will be produced via the solution casting method and, later, the investigation into the physicochemical and antibacterial properties of this film will be done to ensure this biocomposite film of alginate and curcumin meets the ideal requirements of an excellent wound dressing.

2. Materials and Methods

2.1 Materials

Sodium alginate, curcumin, ethanol, and calcium chloride dehydrate were purchased from Sigma Aldrich. Bacterial strains of *Staphylococcus aureus* were used for antibacterial assays. All chemicals used in this study were of reagent grade.

2.2 Film preparation

100 grams of sodium alginate was dissolved in 100 ml distilled water (1wt% sodium alginate) for 30 minutes using magnetic stirrer with the speed of 300 RPM. Simultaneously, 100 grams of curcumin was dissolved in 100 ml of ethanol also using magnetic stirrer with the speed of 300 RPM for one hour.

Both solutions were then mixed according to their own ratio shown in Table 1 and was stirred for another 30 minutes. Then, 20 ml of the mixture was deposited into a petri dish. The mixture was allowed to sit for 5 hours before it was soaked into calcium chloride solution for the cross-linking process. After 24 hours, the excess calcium chloride was removed, and the film was left to dry for another 24 hours.

Table 1 Parameters for film fabrication

Sample	Parameters		
	Alginate solution (ml)	Curcumin solution (ml)	Ratio (curcumin: alginate)
SACu (0.2)	80	20	1:4
SACu (0.4)	60	40	2:3
SACu (0.6)	40	60	3:2
SACu (0.8)	20	20	1:4
SACu (1.0)	100	100	1:1

2.3 Film characterization

2.3.1 Film thickness

The thickness of the films was determined by using a digital micrometer (Mitutoyo, Japan). For each sample, three different thickness values were measured at random locations and their average thickness values were calculated.

2.3.2 Atomic Force Microscopy (AFM) analysis

The surface roughness of the biocomposite film was analysed using an atomic force microscope (AFM) in non-contact mode (Park System XE-100, Korea). The films (1 cm x 1 cm) were placed on the glass slide. The experiment is performed in ambient air at ambient conditions. The surface morphology of the samples is obtained, and the data obtained is processed with the software.

2.3.3 Contact angle analysis

The contact angle of the films was measured using a goniometer (AST VCA Optima, USA). The sample film (1 cm x 1 cm) will be loaded onto the sample stage and the deionized water droplet will be placed on top of the biocomposite film using a 10 L syringe. The water drop image is captured by a camera and the image is used to do an analysis using software..

2.3.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis

To determine the intermolecular interactions within the biocomposite films, the analysis was carried out using ATR-FTIR (Perkin Elmer, USA). The sample is pressed tightly against the crystal plate and the scanning was performed a range of wave numbers from 4000 – 600 cm^{-1}, at a 4 cm^{-1} resolution with 32 scans to generate the FTIR spectrum.

2.3.5 Antibacterial activity

The disc diffusion method, or Kirby-Bauer test, was used to analyse the antibacterial properties of the biocomposite film. For this procedure, the films were cut into 5mm discs and were placed on the microbial plates, which had an adequate amount of MHA medium inoculated with *Staphylococcus aureus, S.aureus.* The process continued with the incubation of the plates for 24 hours at 37°C, and the inhibition zones were recorded after that.

3. Results and Discussion

3.1 Fabrication of the film

The biocomposite films of alginate and curcumin were fabricated with different concentrations of curcumin (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) as shown in Figure 1.

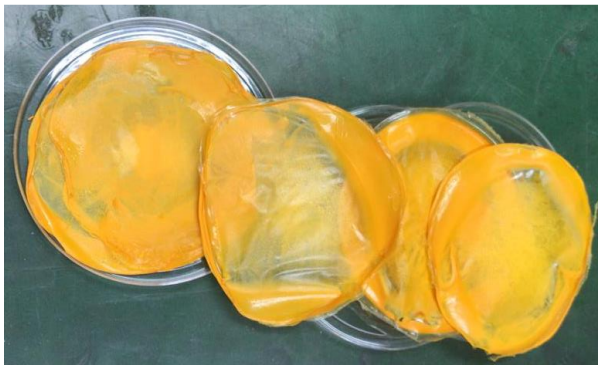

Figure 1 Fabricated film with different concentrations

3.2 Physicochemical characterization of the film

3.2.1 Thickness of the film

The microstructure, molecular orientation, mechanical properties, and moisture absorption of a film are directly related to its thickness. The thickness of the biocomposite film is shown in Table 2.

Table 2 Thickness of film

Sample	Thickness (mm)	Standards deviation (mm)
SA	0.146	0.008
SACu (0.2)	0.146	0.006
SACu (0.4)	0.147	0.004
SACu (0.6)	0.151	0.003
SACu (0.8)	0.151	0.002
SACu (1.0)	0.150	0.005

The increase in curcumin concentrations in the SACu biocomposite films has resulted in an increase in sample film thickness. ver, the difference is only significant after the curcumin concentration at 0.6%. The presence of curcumin at low concentrations in the formulation had no effect on the thickness of the alginate and curcumin biocompoThe thickness of the biocomposite films varied from 0.146 mm to 0.151 mm depending on the concentration. Howesite films. Bashi et al. (2020) discovered that increasing the concentration of curcumin in soybean polysaccharide (SSPS) and titanium dioxide (TiO_2) nanocomposites films increased the thickness of the films [10]. The film thickness difference is probably related to the constituents as well as the hydrophobic molecule content of curcumin.

3.2.2 Surface roughness of the film

Another important characteristic of a wound dressing is its surface roughness. Based on the AFM findings (Figure 2 and Table 3), the SA film has a smooth surface with the lowest Ra value. The incorporation of curcumin in the biocomposite film caused irregular shapes the value of Ra of biocomposite films increased with higher concentration of curcumin and the surfaces of the films become rougher. This meant that with the absence of curcumin, the alginate is able to form uniform structure. However, with the addition of curcumin, the interaction of alginate molecules is altered, resulting in higher value of Ra and the emergence of more noticeable aggregation in the shape of hills

and valleys which might cause by the hydrophobicity of curcumin. Wang et al. (2019) also reported similar results where the sodium caseinate (NaCas)-zein nanocomposite film without curcumin had smoother surface than the film added with curcumin [11]. Other than wettability, rougher surface provides better adhesion since additional surface area offers more space for bonding which facilitates cell adhesion and proliferation.

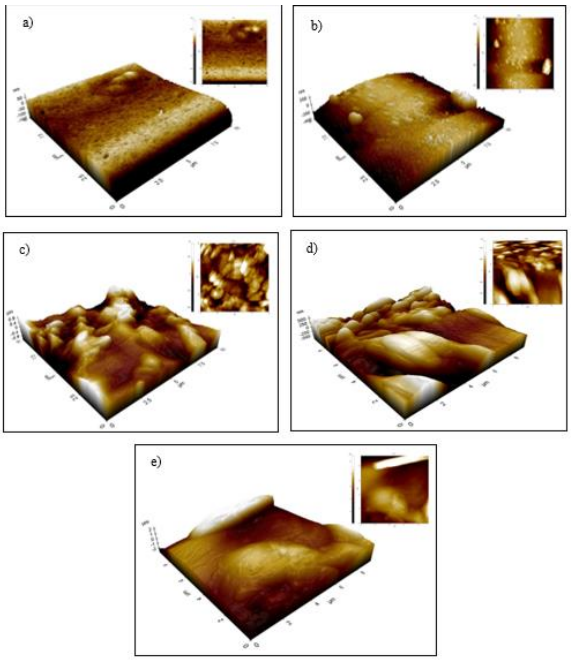

Figure 2 AFM images of a) SA, b) SACu (0.2), c) SACu (0.4), d) SACu (0.6), and d) SACu (0.8)

Table 3 The value of Ra of the films

Sample	R_a (nm)
SA	2.795
SACu (0.2)	6.148
SACu (0.4)	15.617
SACu (0.6)	18.247
SACu (0.8)	23.552

3.2.3 Surface wettability

The absorption capabilities of wound dressings are critical in preventing the buildup of wound exudates, which serve as a breeding ground for bacteria. Low contact angle (< 90°) indicates great wettability. Despite the curcumin being hydrophobic, all the evaluated samples had contact angles less than 90°, indicating that all the films had a hydrophilic surface which means the films are wettable. From the pattern in figure 3, it can be seen when the concentration of curcumin increased, the contact angle insignificantly increased, decreasing the surface wettability. The lipophilic nature of curcumin and the presence of curcumin in the SACu biocomposite film was proved by the decrease in water affinity. Similarly, Reddy & Kim (2019) reported that when curcumin was added to poly-lactic acid (PLA) film, the angle was higher than that of the reference PLA film, and when the curcumin concentration was higher, the contact angle further increased [12]. The observed contact angles matched the AFM findings, which showed that curcumin concentration increased, the surface roughness of the biocomposite film increased significantly.

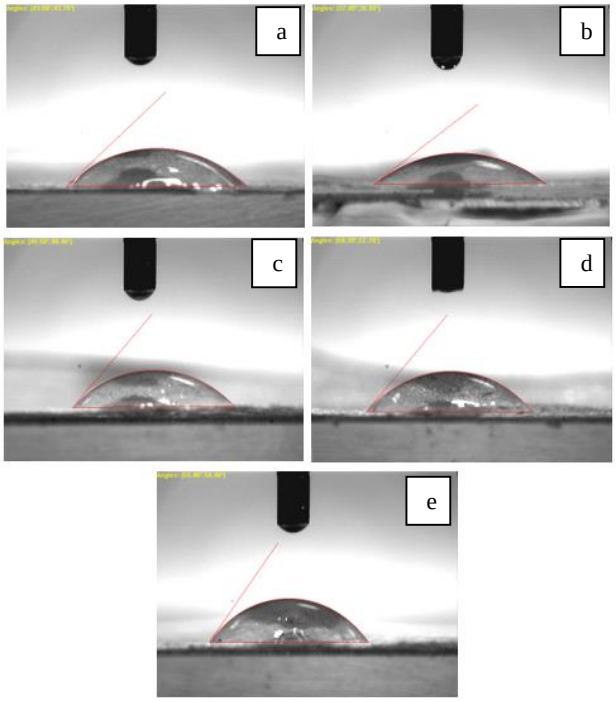

Figure 3 Contact angle images for a) SACu (0.2), b) SACu (0.4), c) SACu (0.6), d) SACu (0.8) and e) SACu (1.0)

Table 4 The value of contact angle

Sample	Contact angle (°)
SACu (0.2)	43.60 ± 3
SACu (0.4)	37.40 ± 3
SACu (0.6)	49.50 ± 3
SACu (0.8)	52.70 ± 3
SACu (1.0)	55.40 ± 3

3.2.4 Chemical structure

The intermolecular reactions within the SACu biocomposite film were analysed and figure 4 displays all the IR spectrum of all biocomposite films of alginate with different concentration of curcumin (0.2, 0.4, 0.6, 0.8, 1.0). The SA film IR spectra shows the stretching vibration at 3250 cm^{-1} due to hydroxyl groups, O-H. Observed peak at 1600 cm^{-1} which attributed to an interaction between carboxyl group and the calcium ions during cross-linking with calcium chloride. The FTIR of sodium alginate also showed peaks at 1025 cm^{-1} due to C-O-C due to alginate's saccharide structure and 950 cm^{-1} due to C-O stretching vibrations of uronic acid in sodium alginate.

The FTIR spectrum of SACu films show features that included both curcumin and alginate composite characteristic bands. The peaks corresponding to these functional groups were observed at 1601–1600 cm^{-1}, 1025–1022 cm^{-1} and 963–961 cm^{-1} respectively, which indicates that the presence of curcumin with different concentration unaltered the major peaks of sodium alginate. The increasing concentrations of curcumin only resulted in minor shift of the main peaks of sodium alginate. The peaks at 3250 cm^{-1} showed a higher shift to 3355-3300 cm^{-1} which indicates the intermolecular reaction of phenolic group of curcumin via hydrogen bonding interaction. The presence of curcumin in the biocomposite films was identified by two new peaks, peak at 1626–1625 cm^{-1} attributed to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character of the inter-ring chain and the sharp peak at 1509–1510 cm^{-1} corresponds to the C=C stretching vibration of the benzene ring.

Another strong appearance at peak 1275–1270 cm^{-1} which corresponds to the C-O stretching frequency of enol [13].

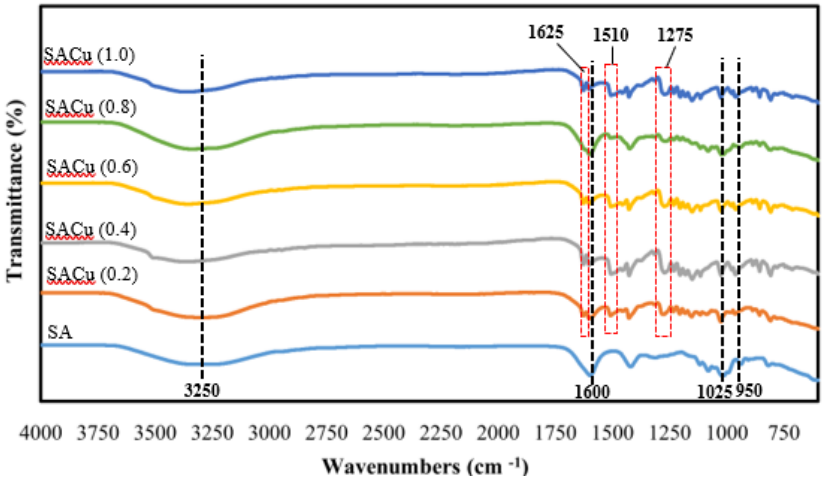

Figure 4 FTIR spectrum of the biocomposite films

3.3 Antibacterial properties

From Figure 5, there was no inhibition zone around the SACu films on the agar surface after 24 hours of incubation, while there were inhibition zones around the controlled antibacterial films. This shows that the fabricated biocomposite SACu films do not inhibit antibacterial properties to be used on wounds. The possible reason for the biocomposite films failing to have antibacterial activities is that the biocomposite films probably did not have enough concentrations of curcumin, as the ones that have been tested for antibacterial assessments were just 0.2%, 0.4%, and 0.6% sample films. This is similar to the results from studies by Abou et al. (2018) who reported that only after 300 L of extracted curcumin was added to the agar surface showed the defined zone of inhibition against *S.aureus* and the inhibition zones increased with the addition of the volumes of extracted curcumin [14]. The lack of antibacterial effect of the biocomposite film of alginate and curcumin may also be due to poor curcumin handling during biocomposite film preparation, as curcumin is very sensitive and has a high degradation rate. investigations and developments must be made to enhance the antibacterial activities of the alginate and curcumin biocomposite films by using various other methods such as the capsulating technique.

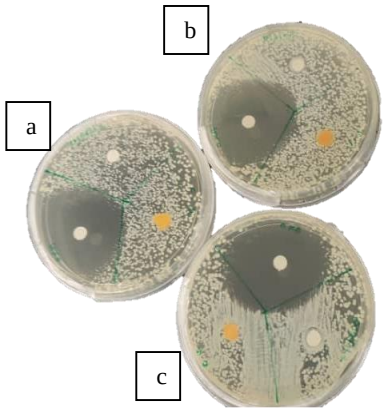

Figure 5 Inhibition zone for a) SACu (0.2), b) SACu(0.4), and c) SACu(0.6)

3. Conclusion

In the present study, a potential biocomposite film of alginate and curcumin for wound healing applications was successfully fabricated via solution casting method. The FTIR spectrum of the sample revealed the successful intermolecular interactions between curcumin and alginate as the result

displayed all characteristics bands of pure sodium alginate when the curcumin was added, and a few new peaks appeared because of curcumin's characteristic bands. On the other hand, when the concentration of curcumin in the SACu biocomposite film increased, so had the thickness and contact angle of the film. The surface of the films also became rougher with the increasing curcumin's concentrations. This is all associated to one of curcumin's properties, which is that it is hydrophobic. However, the SACu biocomposite films are still hydrophilic due to the alginate based and the rougher surfaces provide good adhesion for wound site. Antibacterial testing was also performed on the sample and the fabricated biocomposite film had failed to inhibit bacteria growth. More research is needed for better understanding prior to developing curcumin and alginate biocomposite film as a potential wound dressing.

Acknowledgement

The authors would like to thank the Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia for the support in implementing the research.

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