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PVA-PEG Hydrogel Incorporated with Cellulose Nanofibril of Oil Palm Empty Fruit Bunches and Antibacterial Agent Curcumin

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ABSTRACT

Introduction: The compelling characteristics of hydrogel films, resembling biological tissues, have sparked significant interest for their use in wound healing dressings.

Materials and methods: Cellulose nanofibrils (CNFs) and antibacterial agent of curcumin was incorporated into polyvinyl alcohol (PVA)-polyethylene glycol (PEG) hydrogel prepared via few cycles of freeze-thaw methods. The CNFs were extracted from oil palm empty fruit bunches (OPEFB) using alkaline-deep eutectic solvent (alkaline-DES) assisting with ultrasonication. The inclusion of CNFs and curcumin were optimized by varying their concentrations and moisture retention content (MRC) was determined as a response.

Results: The PVA-PEG/CNF-curcumin hydrogel achieved a 44.84% MRC via an optimal hydrogel composition comprising 6% (v/w) CNF and 5% (v/w) curcumin. Other physio-chemical properties of the developed hydrogel such as swelling behaviours, water vapor transmission rate (WVTR), hydrogel porosity, chemical structural, and antimicrobial resistance were determined as well to observe the effect of incorporating of CNFs and curcumin. The optimized PVA-PEG/CNF-curcumin hydrogel formulation demonstrated a swelling capacity of 26.44%, enhanced porosity of 48%, and a WVTR of 76.73 g/m²h, showed its potential as a promising dressing material with improved characteristics. The PVA-PEG/CNFs-curcumin hydrogel was observed to have high moisture retention content and demonstrated good resistance to gram-positive bacteria (*B. subtilis*) and lower resistance to gram-negative bacteria (*E. coli*).

Conclusion: In conclusion, the incorporation of CNFs and curcumin into PVA-PEG hydrogel demonstrated promising characteristics, highlighting its potential as an effective and versatile wound healing dressing with notable antimicrobial properties.

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Introduction

Over the years, polyvinyl alcohol (PVA) hydrogel had been applied widely in the in the medical field specifically for creating artificial organs, contact lenses, medication delivery systems, and wound dressings (Kamoun et al., 2021). PVA's similar physical characteristics make it compatible with human tissue. Due to its structure, which can absorb protein molecules, interact with minimum cell adhesion, and have no harmful effects, PVA membranes had been widely developed for biomedical purposes (Chen et al., 2017; Kamoun et al., 2021). However, PVA hydrogel still had some limitations that need to be improved and these limitations had restricted its usage in several medical and other industrial applications (Cui et al., 2021). Due to that, further studies are required to investigate its physiochemical properties like texture and swell ability, mechanical strength and find ways to improve it. While polyethylene glycol (PEG) is also another non-toxic polymer which is highly recognized for its high chain flexibility and superior temperature resistance, facilitating the development of hydrogels with relatively high swelling capacity (Cui et al., 2021).

Thus, in this study, the PVA-PEG hydrogel was reinforced with cellulose nanofibrils (CNFs) from empty fruit bunch (EFB) and incorporated with a natural antibacterial agent, curcumin. Previous studies reported that inclusion of nanomaterials specifically CNFs is expected to improve the mechanical properties of the PVA-PEG hydrogel (Butylina et al., 2016, 2020). A high crystallinity and aspect ratio of CNFs as well as apparently biocompatible nanomaterials make them attractive nanofillers in synthesizing the hydrogel with improved physical properties. Few studies on polymeric hydrogels incorporated with nanofillers showed that the incorporation of nanocellulose fibers can increase physical properties, thermal properties, and barrier properties (Chen et al., 2017; Cui et al., 2021; Kamoun et al., 2021). Furthermore, the incorporation of natural antimicrobial agent, curcumin may exhibit antibacterial properties which are important for polymeric hydrogel used for wound healing in preventing any wound infections (Alven et al., 2020).

Hence, the current study focuses to develop and optimize appropriate compositions of CNFs and curcumin in fabricating the PVA-PEG hydrogel via freeze-thaw process by response surface methodology (RSM). Physio-chemical properties of the optimized hydrogel was determined specifically moisture retention content (MRC), swelling behaviors, water vapor transmission rate (WVTR), hydrogel porosity, chemical structural as well as antimicrobial resistance to gram-positive bacteria *Bacillus subtilis* (*B. Subtilis*) and gram-negative bacteria *Escherichia coli* (*E. coli*).

Materials and methods

Chemicals and Biomass Raw Materials

The oil palm empty fruit bunches (OPEFB) fibers were collected from Kilang Sawit FELCRA Maran (Pahang, Malaysia). After cleaning the collected OPEFB with tap water, it was cut into small pieces, dried, and sieved to 100-micron size before kept in a sealed container at room temperature. All chemicals were purchased from Sigma-Aldrich, USA except for curcumin. All chemicals are analytical grades: 98% sodium hydroxide pellet (NaOH), 35% hydrogen peroxide (H_2O_2), oxalic acid dihydrate (OAD) (≥ 99 %), choline chloride (ChCl) (≥ 98 %), polyvinyl alcohol (PVA; M_W 89,000-98,000; 99% hydrolysed), polyethylene glycol (PEG; M_W 3350), and dimethyl sulfoxide (DMSO; ≥99.5%). Curcumin powder was used as an antibacterial agent for this research and was purchased from Merck.

Extraction of CNF from OPEFB

The extraction of CNFs from OPEFB followed the method described by Jafri et al. (2024), but with several modifications. The grounded EFB fibers were washed with hot distilled water at 80 °C for 1 h and dried in oven at 50 °C until constant weight achieved. Then, the extraction and purification of cellulose from EFB fibers comprised mainly of three steps: alkaline treatment, bleaching, and DES treatment. First, the EFB fibers were dissolved in a 4% (w/v) sodium hydroxide solution with a 1:30 (w/v) fiber-to-solution ratio. The mixture was agitated at 85 °C for 2 h. After that, the fibers were continuously rinsed in deionized water. After that, the pretreated fibers were dissolved in DES of ChCl-OAD at a fiber-to- solution ratio of 1:10 (w/v). The DES ratio of ChCl: OAD is 1:2. The suspension was then filtered, neutralized with deionized water, and dried for 24 h in an oven at 50 °C. The fibers were then bleached using 10% (w/v) hydrogen peroxide at 90 °C for 3 h with a 1:30 (w/v) fiber to solution ratio. This step was repeated three times until the fibers turned white. The obtained fibers were filtered, neutralized with deionized water, and oven-dried at 60 °C until their weight remained consistent.

Ultrasonicating the Extracted Cellulose

A specific amount of the extracted cellulose was mixed in deionized water (50 mL) to achieve concentration of 10% wt. The suspension was homogenized for 2 h ultrasonication at 80% (280 W) sonication amplitude to individualize CNFs. The ultrasonic homogenizer (FisherbrandTM Model 705 Sonic Dismembrator) fitted with a 1/8" sonication probe was used. The suspension samples were placed in an ice bath to prevent any damages to the samples as this process may generate some heat.

Preparation of PVA-PEG-CNF Hydrogel with Curcumin

The hydrogel preparation method was adopted from Altaf et al. (2021), with some modification. 15 (w/w%) of polyvinyl alcohol (PVA) was heated into 80 mL deionized water mixed with 20 (w/w%) of DMSO solution and stirred for 50 mins at 80°C. After that, 6 (w/w%) of polyethylene glycol (PEG) was added into the PVA solution, heated at 80 °C and stirred for 45 mins. Subsequently, varied amount of CNF ranging from 1 to 6 (w/w%) were added into the PVA-PEG solution, heated 80°C and stirred for 45 mins. Then, different contents of curcumin ranging from 0 to 5 (w/w%) were added in the PVA-PEG/CNF solution with the PVA crosslinking agent. Details of the optimization of PVA-PEG/CNF hydrogel with curcumin are shown in Section 2.5. The chemical crosslinking agent solution was prepared by adding 0.5 ml of glutaraldehyde (GA) and 0.05 ml of HCl in 10 mL of ethanol (Altaf et al., 2021). This solution was added to the PVA-PEG/CNF solution with constant stirring. Then 4 mL of glycerin was added with constant stirring and the solution was sonicated for 2 h. The hydrogel underwent three freeze-thaw cycles, each of which involved reducing the temperature to -20 °C for 24 hours and thawing for 4 h at room temperature. The hydrogels were then kept at 4 °C until further analysis.

Optimization of CNF and curcumin concentrations in PVA-PEG/CNF Hydrogel

The optimal formulation of the PVA-PEG hydrogel with constant concentration of PVA and PEG and varied concentration CNF (A) and curcumin (B) ranging from 1 to 6 (w/w%) and 0 to 5 (w/w%) respectively. Central composite design (CCD) using Design-Expert version 13.0.21.0 software (Stat-Ease Inc., Minneapolis, MN) was used and moisture retention content (MRC) is the response. Table 1 shows the CCD experimental design. The produced hydrogel was denoted as PVA-PEG/CNF-curcumin.

Moisture Retention Content

Prepared hydrogel membranes were cut into equal pieces of 2 cm × 2 cm with a thickness of 0.3 cm and then weighed. These samples were then placed inside an oven for 6 h at 40 °C. Later, they were removed from the oven and weighed again. Equation 1 was used to determine the moisture retention content (MRC) (Altaf et al., 2021).

$$MRC\ (\%) = (W_f/W_i) \times 100 \quad (1)$$

Where, W_i is the initial weight and W_f is the weight of sample after 6 h of heating at 40 °C.

Swelling Behavior Test

The hydrogel was cut into equal pieces of 2 cm × 2 cm with a thickness of 0.3 cm and then weighed to measure the swelling behavior. The hydrogel samples were submerged in distilled water at room temperature for 24 h until the hydrogel reached its equilibrium swelling state. The hydrogel then be promptly removed, and any leftover solvent on the surface was swiftly blotted with absorbent paper before being weighed once more. Equation 2 used to calculate swelling behavior (Altaf et al., 2021).

$$\text{Swelling ratio}\ (\%) = \frac{(W_s - W_d)}{W_d} \times 100 \quad (2)$$

Here, W_s is the weight of swelled hydrogel and W_d is the weight of dried hydrogel.

Table 1: CCD for each variable along with its corresponding ranges.

Coded	Variables	Levels		
		-1	0	+1
A	CNF concentration	1	3.5	6
B	Curcumin concentration	0	2.5	5

Antibacterial Test

The antibacterial activity was determined via disc diffusion method as described by Altaf et al. (2021), with little modifications to evaluate the effectiveness of generated hydrogels against bacteria. Several different bacterial strains were used for antibacterial studies such as *Bacillus subtilis (B. subtilis)* and *Escherichia coli (E. coli).* Inoculum was prepared by transferring a loopful of bacteria cells from the stock cultures into sterile Luria-Bertani (LB) broth placed in a 10 ml centrifuge tube which them kept at 4 °C. Then, this bacterial culture was incubated at 37 °C for 24 hr in an incubator shaker at 150 rpm. Then, 0.1 mL of overnight cell culture was inoculated on LB agar plate and were spread evenly on the surface of agar plates and left to dry for few minutes. A UV-sterilized equal-sized piece (1 cm × 1 cm) with a thickness of 0.3 cm of hydrogel sample was deposited on the bacteria-containing agar plates, and then incubated for 24 hr at 37 °C. Bacterial growth inhibition was determined by measuring the diameter of the inhibition zones around the hydrogel sample. Chlorohexidine (0.05%) and chlorohexidine gluconate (0.05%) were employed as controls.

Water Vapor Transmission Rate Measurement

To determine WVTR, 10 mL distilled water was poured into media glass bottles (small brown bottles). Measured the diameter of the mouth opening of the bottles. These bottles were covered with hydrogel membranes, wrapped through Teflon tape and then were weighed. These bottles were located at 50 °C inside an oven for 1 day. After 1 day, they were weighed again and WVTR (g/m2h) was evaluated using Equation 3 (Altaf et al., 2021).

$$\text{WVTR} = (W_i - W_t)/(A \times 24) \times 10^6 \qquad (3)$$

Where, A is the area of the round opening of the bottle, W_i is the mass of bottle before heating and W_t is the weight of bottle after heating.

Hydrogel Membrane Porosity

The hydrogel samples were immersed into ethanol until they got flooded. Ethanol was used to wet the sample and immerse it. Equation 4 was used to determine the porosity (Altaf et al., 2021).

$$\varphi\ (\%) = (W_f - W_i)/(\rho V_f - \rho V_i) \times 100 \qquad (4)$$

Where, W_1 and W_2 specify the weight of samples earlier and later having absorption in ethanol, respectively. V_1 is the volume of ethanol before absorption, V_2 is the volume of ethanol after absorption and ρ is density is the density of alcohol at room temperature.

Fourier Transform Infrared Spectroscopy (FTIR)

The structural changes that appeared in polymeric hydrogel membranes were evaluated by FTIR. The dried and impurity-free samples were subjected to FT-IR. The spectra were recorded by an FT-IR spectrometer equipped with an attenuated total reflection (ATR) unit in the range of 400–4000 cm^{-1}. The hydrogel samples were put directly into the FT-IR machine. All analysis was done at ambient temperature.

Results and Discussion

Statistical Design and Optimization Analysis

The impact of CNF and curcumin concentrations on the MRC of produced PVA-PEG/CNF-curcumin hydrogel were examined via RSM. Based on RSM, a total of 13 experimental runs were studied, and the experimental findings are displayed in Table 2.

Moreover, the experiment employed analysis of variance (ANOVA) to assess both the efficacy and significance of the created model. ANOVA, a statistical technique, was utilized to validate the model by gauging the significance of various factors and interactions within it (Rahmi et al., 2020; Rodrigues et al., 2019). The determination of the model's meaningfulness relied on the examination of the probability value, commonly known as the p-value. A model attains significance when its p-value is below 0.05, indicating a probable real impact on the studied system. The p-value played a crucial role in detecting and eliminating any irrelevant interactions or factors, ensuring the accurate representation of relationships within the data (Rahmi et al., 2020; Rodrigues et al., 2019).

The model attained was significance, evident in its low p-value ($p < 0.0001$) and an F-value of 33.91. Additionally, ANOVA affirmed that all terms (A-CNF concentrationand B-curcumin concentration) demonstrated p-values below 0.05, signifying their significance as model terms. This outcome establishes that variables such as CNF and curcumin concentrations significantly impact the MRC of the hydrogel. Furthermore, the evaluation of the model's fit to experimental data can be carried out using the regression coefficient R^2, adjusted R^2, and estimated R^2 (Bacha, 2022). A strong alignment between the model and the experimental data is indicated when these coefficients approach a value of 1 (Rahmi et al., 2020). Notably, the substantial R^2 of 0.8715, approaching 1, provides evidence that the model fits the experimental data effectively. Moreover, the predicted R^2 of 0.7255 aligns well with the adjusted R^2 of 0.8458, differing by less than 0.02, affirming the model's accurate predictions (Thakur et al., 2020). The extent to which the developed model fails to predict variance is elucidated by the Lack-of-fit. The insignificance in the Lack-of-fit underscores the adequacy of the fitted model, indicating a robust correlation between process variables and output response (Shitole et al., 2019; Thakur et al., 2020).

Table 2: Experimental design.

Run	CNF concentration [A] (% v/w)	Curcumin concentration [B] (% v/w)	MRC (%)
1	3.5	2.5	41.84
2	3.5	2.5	40.39
3	3.5	0	39.21
4	1	0	33.77
5	1	5	40.56
6	1	2.5	38.38
7	3.5	5	43.29
8	6	2.5	42.59
9	6	0	41.79
10	3.5	2.5	41.67
11	3.5	2.5	42.31
12	3.5	2.5	41.56
13	6	0	44.84

The examination of the favorable statistical characteristics of the developed model can include parameters such as adequate precision, standard deviation, and the coefficient of variation (C.V.%). The model's adequate precision stands at 19.72%, indicating a sufficient signal for the response surface area (Rahmi et al., 2020; Rezvanian et al., 2017). A preferred response is indicated by an adequate precision value exceeding 4, denoting a suitable signal response to the process and its ability to move within the design space. Additionally, the model exhibits a favourable standard deviation of 1.07%, considered advantageous as it falls below 3%. A lower standard deviation signifies a close correspondence between the predicted result and the actual response (Rodrigues et al., 2019). The coefficient of variation (C.V.%) serves to assess the accuracy and validity of the investigations. The determined C.V.% level is 2.61%, less than the recommended threshold of 10%. Lower C.V.% values are preferred, as they indicate a more precise and rational experimentation approach (Altaf et al., 2021). Figure 1 illustrated the three-dimensional response surface graph of hydrogel MRC values.

According to Figure 1, the optimal formulation for the PVA-PEG/CNF-curcumin hydrogel resulted in a 44.84% MRC, achieved with 6% (v/w) of CNF and 5% (v/w) of curcumin. The enhanced MRC of the PVA-PEG/CNF-curcumin hydrogel at 44.84% can be attributed to the synergistic effects of incorporating CNF and curcumin into the PVA-PEG hydrogel matrix. The introduction of CNF, known for its high aspect ratio and hydrophilic nature, likely contributes to increased water absorption and retention within the hydrogel structure. The introduction of CNF into the PVA-PEG hydrogel structure creates a network with a large surface area, providing numerous sites for water molecules to interact and form hydrogen bonds (Rodrigues et al., 2019). The intertwined network of CNF within the polymer matrix may create capillary forces that enhanced water retention, preventing its easy evaporation and promoting sustained moisture content. This increased interaction facilitated water absorption and retention within the hydrogel, contributing significantly to the observed rise in MRC (Ahmed et al., 2018).

Furthermore, the inclusion of curcumin, a natural compound found in tumeric has antioxidant and anti-inflammatory properties thus were able to enhance its features in retaining moisture properties (Alven et al., 2020). Curcumin's hydrophilic characteristics may facilitate water absorption, while its interaction with the polymer chains could potentially enhance the hydrogel's overall water retention capacity (Alven et al., 2020). The combined effects of CNF and curcumin thus create a hydrogel formulation with improved moisture retention, offering potential applications in areas where maintaining high levels of hydration is crucial, such as wound healing (Alven et al., 2020; Miah et al., 2017).

Polyethylene glycol (PEG) further enhances the hydrogel's performance in moisture retention. PEG is a hydrophilic polymer that is well-known for its water-absorbing capabilities (Ahmed et al., 2018). By incorporating PEG into the hydrogel matrix, it provides additional sites for water molecules to interact and be retained within the structure. PEG's ability to form hydrogen bonds with water molecules and its compatibility with the other components in the hydrogel contribute to the overall enhancement of the hydrogel's water retention capacity (Cui et al., 2021). Therefore, the combined effects of CNF, curcumin, and PEG create a multifaceted hydrogel system with improved moisture retention, holding significant promise for applications in biomedical and cosmetic fields (Lv et al., 2019).

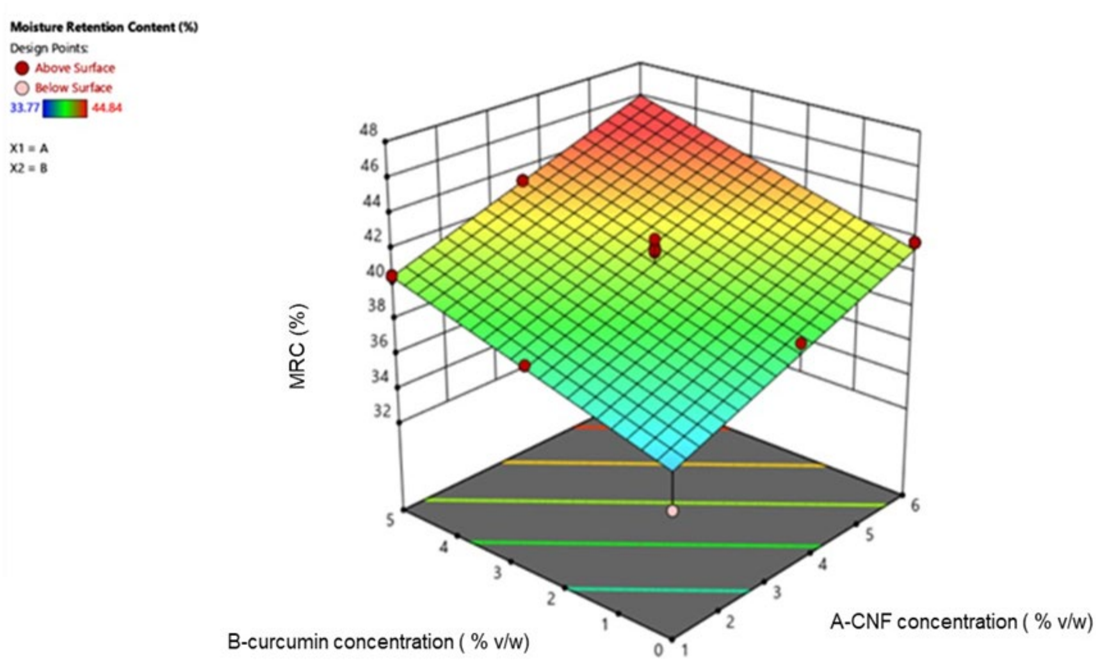


Figure 1: The surface plot showed the effect of CNF and curcumin concentrations on the MRC of hydrogel.

Swelling Behaviors of Hydrogel

The hydrogels, upon absorbing water, underwent swelling, displaying characteristics akin to biological tissue. This behavior is crucial in biomedical applications, particularly in wound care, where the hydrogel's ability to absorb and retain liquids on the wound surface is a key determinant of its effectiveness as a dressing material (Rahmi et al., 2020). The swelling capacity of a hydrogel is indicative of its ability to accommodate and retain moisture, a critical feature for promoting wound healing and maintaining a conducive environment for tissue repair (Lv et al., 2019). In the case of the optimized PVA-PEG/CNF-curcumin hydrogel formulation, the observed swelling capacity of 26.44% underscores its potential as a promising dressing material. This swelling capability indicates that the hydrogel can efficiently absorb and retain a significant amount of liquid, making it well-suited for applications where moisture retention on the wound surface is essential (Lv et al., 2019).

The swelling capacity of the PVA-PEG/CNF-curcumin hydrogel, reaching 26.44%, indicated a significant improvement and potential for biomedical applications. The incorporation of PEG into the hydrogel formulation played a crucial role in enhancing its swelling properties. PEG is a hydrophilic polymer known for its water-absorbing capabilities. By introducing PEG into the PVA-PEG hydrogel matrix, additional hydrophilic sites are provided, promoting increased water absorption. The intermolecular interactions between PEG and water molecules, facilitated by hydrogen bonding, contribute to the hydrogel's ability to swell and retain moisture efficiently (Cui et al., 2021). This synergistic effect results in a hydrogel with a substantial swelling capacity, making it well-suited for applications where controlled hydration and moisture retention are vital, such as in wound dressings (Ahmed et al., 2018).

Furthermore, the integration of CNF into the hydrogel formulation contributes to its swelling behavior. CNF, with its high surface area and hydrophilic nature, creates a network within the hydrogel matrix that enhances water absorption. The porous structure formed by CNF provides ample spaces for water molecules to be absorbed, leading to increased swelling capacity (Butylina et al., 2016, 2020). The combination of CNF and PEG creates a dual mechanism for water absorption, optimizing the hydrogel's ability to swell while maintaining structural integrity. The role of curcumin in the hydrogel formulation also adds to its swelling potential. Curcumin, being hydrophilic, contributes to the overall water-absorbing capabilities of the hydrogel. Additionally, the interactions between curcumin and the polymer chains further influence the hydrogel's structure, making it conducive to water absorption and retention (Alven et al., 2020). The collective impact of PEG, CNF, and curcumin creates a multifaceted hydrogel system with a notable swelling capacity, showcasing its potential for applications in wound care and other biomedical fields.

Hydrogel Membrane Porosity

The porosity of a hydrogel, indicating the extent of voids or empty spaces within its structure, stands as a vital parameter in the realm of wound dressings (Altaf et al., 2021). Its significance lies in its direct impact on the hydrogel's capacity to absorb and retain crucial fluids like wound exudate and water, pivotal for establishing a conducive, moist wound environment conducive to healing. An appropriate level of porosity allows the hydrogel to efficiently absorb these fluids, preventing leakage and maintaining the necessary equilibrium between moisture management and fluid retention (Altaf et al., 2021). Furthermore, porosity facilitates the exchange of oxygen and nutrients, which are crucial for tissue regeneration, and supports the removal of waste materials from the wound site (Rahmi et al., 2020). Hence, maintaining an optimal level of porosity emerges as a critical factor to ensure the hydrogel effectively plays its role in the context of wound care.

The porosity of the PVA-PEG/CNF-curcumin hydrogel was 48%, indicates a notable increase resulting from the incorporation of PEG, CNF, and curcumin into the PVA-based hydrogel matrix. Porosity is a critical parameter as it reflects the proportion of empty spaces or voids within the hydrogel structure, influencing its ability to absorb and retain fluids (Altaf et al., 2021). In this case, the inclusion of PEG, known for its hydrophilic nature, likely contributes to enhance water absorption capacity, leading to increase porosity (Ahmed et al., 2018). Additionally, CNF, with its high surface area and hydrophilicity, may play a role in creating a porous network within the hydrogel, further contributing to the observed increase in porosity (Butylina et al., 2016, 2020). Curcumin, being hydrophilic as well, may also contribute to the hydrogel's porous structure, although its specific role in porosity enhancement may depend on its interaction with other components (Alven et al., 2020).

In wound dressings, the optimal porosity range of 30% to 40% is often recommended (Altaf et al., 2021). This range is considered suitable for promoting a moist wound environment, ensuring effective fluid absorption, and facilitating gas exchange for tissue regeneration. Consequently, while the observed porosity of 48% in the PVA-PEG/CNF-curcumin hydrogel surpasses the typical range for hydrogels utilized in wound dressings, this higher porosity level presents potential advantages, including heightened fluid absorption and improved gas exchange (Baghaie et al., 2017). These attributes may prove beneficial in specific wound healing scenarios. However, it's also crucial to balance these advantages with the need for structural integrity and mechanical stability, as excessively high porosity might compromise the hydrogel's ability to maintain its form and adhere to the wound surface (Altaf et al., 2021).

WVTR of Hydrogels

The Water Vapor Transmission Rate (WVTR) of hydrogels is a critical parameter, particularly in the context of wound dressings. WVTR measures the ability of a material, such as a hydrogel, to allow the passage of water vapor through its structure. This property is crucial for wound dressings as it directly influences the regulation of the wound environment (Altaf et al., 2021). An optimal WVTR is essential to strike a balance between preventing excessive moisture buildup, which could lead to maceration and bacterial growth, and promoting sufficient moisture to facilitate the healing process (Baghaie et al., 2017). Hydrogels with an appropriate WVTR offer a breathable and permeable barrier that allows for effective gas exchange, ensuring the removal of excess moisture and promoting a conducive environment for tissue regeneration. In wound care, maintaining an ideal WVTR is thus paramount for supporting the healing process, preventing complications, and enhancing overall wound management (Altaf et al., 2021; Lin et al., 2019).

The WVTR of the produced PVA-PEG/CNF-curcumin hydrogel, measuring at 76.73 g/m^2h, reflected the material's ability to allow the passage of water vapor. This substantial WVTR suggested that the incorporation of PEG, CNF, and curcumin into the PVA-based hydrogel has significantly enhanced its moisture permeability. PEG, being a hydrophilic polymer, likely contributed to increased water vapor transmission by promoting the absorption and movement of moisture through the hydrogel matrix. This enhanced hydrophilicity facilitates efficient water vapor transfer, leading to the observed higher WVTR (Ahmed et al., 2018; Cui et al., 2021).

The addition of CNF further augmented the WVTR of the hydrogel. The high surface area and hydrophilic nature of CNF create a network within the hydrogel, potentially forming pathways for water vapor diffusion. This network structure, coupled with the hydrophilic interactions between CNF and water molecules, enhances the overall permeability of the hydrogel to water vapor (Butylina et al., 2016; Carating et al., 2019). Moreover, the incorporation of curcumin, known for its hydrophilic properties, may contribute to the hydrogel's increased WVTR by influencing the interactions between water molecules and the polymer matrix, further facilitating moisture transmission (Alven et al., 2020; Miah et al., 2017).

This elevated WVTR is advantageous for enhancement moisture management by facilitating the efficient removal of excess water vapor from the wound site. This is essential in preventing the accumulation of moisture, which, if not properly controlled, can lead to complications such as maceration and bacterial growth. Moreover, an elevated WVTR supports a breathable environment, enabling the exchange of gases such as oxygen and carbon dioxide. This is vital for tissue regeneration, as it ensures an optimal oxygen supply to the wound area, fostering a conducive milieu for the healing process (Altaf et al., 2021; Carating et al., 2019). The combination of PEG, CNF, and curcumin has synergistically influenced the WVTR, making the PVA-PEG/CNF-curcumin hydrogel a promising candidate for applications where controlled moisture permeability is essential.

Anti-Bacterial Activity

The PVA-PEG/CNF-curcumin hydrogel showed robust resistance against gram-positive bacteria, specifically *B. subtilis.* However, the hydrogels showed comparatively lower resistance when tested against gram-negative bacteria of *E. coli.* This distinction in antibacterial efficacy between gram-positive and gram-negative bacteria underscores the specific impact of the hydrogel formulation on different bacterial types. The observed antibacterial properties suggest that the integration of curcumin, combined with the structural support provided by PVA-PEG/CNF, contributed to the hydrogel's ability to combat certain bacterial strains effectively.

A more pronounced and larger inhibitory zone on the *B. subtilis* plate compared to the *E. coli* plate were observed. This observation suggested that the developed PVA-PEG/CNF-curcumin hydrogels exhibited a stronger inhibitory effect against *B. subtilis* than against *E. coli.* The size of the inhibition zone serves as a visual representation of the antibacterial activity, with a clearer and larger zone correlating with more potent inhibitory effects (Alven et al., 2020; Miah et al., 2017). The PVA-PEG/CNF-curcumin hydrogel exhibited an inhibition zone measuring 16.0 ± 0.5 cm against *B. subtilis,* whereas no inhibition zone was observed for *E. coli*.

The antibacterial activity of curcumin within the PVA-PEG/CNF-curcumin hydrogel against *B. subtilis* can be attributed to the inherent properties of curcumin, a natural polyphenolic compound known for its antimicrobial effects. Curcumin has demonstrated antimicrobial activity against a broad spectrum of bacteria, including gram-positive strains like *B. subtilis*. Its mechanism of action involves disrupting bacterial cell membranes, interfering with cellular processes, and inducing oxidative stress, leading to the inhibition of bacterial growth. The incorporation of curcumin into the hydrogel matrix enhances its antibacterial properties, creating an environment where the release of curcumin molecules actively hinders the proliferation of *B. Subtilis* (Alven et al., 2020; Miah et al., 2017). The observed inhibition zone against *B. subtilis* reflected the successful deployment of curcumin's antibacterial potential within the hydrogel.

On the other hand, the absence of an inhibition zone against *E. coli* may be attributed to the intrinsic differences in the cell wall structure and membrane properties between gram-positive and gram-negative bacteria. Gram-negative bacteria possess an outer membrane that acts as a barrier, making them less susceptible to certain antimicrobial agents (Altaf et al., 2021). Additionally, the hydrogel's components, such as PVA-PEG and CNF, may interact differently with gram-negative bacteria, affecting the release or efficacy of curcumin in hindering their growth. The lack of resistance against *E. coli* might also be influenced by the specific concentration of curcumin within the hydrogel, as gram-negative bacteria often require higher concentrations of antibacterial agents for effective inhibition (Alven et al., 2020; Miah et al., 2017).

Furthermore, the variable responses against different bacterial strains highlight the selective nature of the PVA-PEG/CNF-curcumin hydrogel's antibacterial activity. While curcumin contributes significantly to the inhibition of gram-positive bacteria like *B. subtilis*, the observed lack of resistance against *E. coli* emphasizes the need for a nuanced understanding of the interactions between hydrogel components and bacterial species. Table 3 shows the summary of the antibacterial activity of the hydrogel compared to the controls. The chlorohexidine (0.05%), chlorohexidine gluconate (0.05%), water and curcumin were used as controls.

Table 3: Antibacterial activity data of hydrogel.

Compound	Inhibition zone diameter (mm)	
	E. coli	*B. subtilis*
Hydrogel (6% CNF, 5% curcumin concentration)	NA	16.5 ±0.5
Control 1 (Chlorohexidine 0.05%)	NA	17.0 ± 0.5
Control 2 (Chlorohexidine Gluconate 0.05%)	15.0 ± 0.5	16.0 ± 0.5
Water	NA	NA
Curcumin (5% Concentration)	NA	6.0 ± 0.5

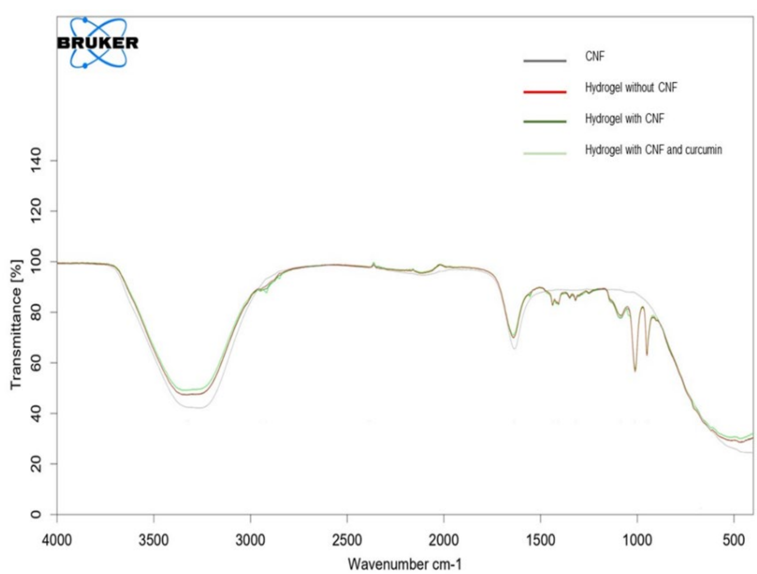
FTIR Analysis

The CNF exhibited peaks at 3262.62 cm^{-1}, indicating the presence of free hydroxyl groups. Additionally, for the hydrogel membranes, both with and without CNF, distinct peaks at 3321.96 cm^{-1} and 3320.04 cm^{-1}, respectively, were observed, signifying the existence of free hydroxyl groups. When the hydrogel membrane contained a 5% (v/w) concentration of curcumin, a robust peak at 3333.33 cm^{-1} was evident, indicating the presence of hydroxyl groups. This observation suggests that the introduction of curcumin into the hydrogel matrix results in a distinct alteration in the FTIR spectra, specifically in the hydroxyl group region, potentially signifying interactions between curcumin and the hydrogel components.

As indicated by Figure 2, the hydroxyl groups of PVA and PEG, engaged in hydrogen bonding interactions, exhibit -OH bond vibrations within the range of 1100-1450 cm^{-1} (Bacha, 2022). Notable peaks observed at approximately 1438 cm^{-1} and 2916 cm^{-1} signify the stretching vibrations of -CH and CH_2, respectively, with PVA being the primary contributor to these vibrations (Li et al., 2019). Additionally, the presence of both C=O and C=C bonds, inherent in the primary components of PVA, PEG, and LG, is evidenced by peaks at 1600 and 1800 cm^{-1} (Saleem & Saeed, 2020). It is important to highlight that the broadest band observed in each spectrum, spanning between 3200 and 3400 cm^{-1}, results from the stretching vibration of -OH (hydroxyl) bonds present in PVA and PEG (Bialik-Was et al., 2021; Cui et al., 2021).

As indicated by Figure 2, the inclusion of cellulose in the hydrogel is confirmed by the emergence of a distinct peak at approximately 1011 cm^{-1}, indicating the C-O and C-H stretching vibration (Yang et al., 2021). Notably, several significant peaks are detected at lower wavenumbers. The $-CH_2-$ group within the aliphatic PVA chain gives rise to C-H bonds, as affirmed by deformation vibrations in the 1430–1440 cm^{-1} region (de Lima et al., 2020).

Figure 2 depicted the Fourier transform infrared (FTIR) images of hydrogels.



Conclusion

This study aimed to develop a polyvinyl alcohol-polyethylene glycol (PVA-PEG) hydrogel reinforced with cellulose nanofibers (CNF) derived from oil palm empty fruit bunches (OPEFB), incorporating with curcumin as an antibacterial agent potentially to be used as wound dressings. The produced PVA-PEG/CNF-curcumin hydrogel showed the significant enhancements in MRC, WVTR, swelling capacity and hydrogel membrane porosity in the optimized hydrogel composition of 6% (v/w) CNF and 5% (v/w) curcumin. The presence of curcumin in the produced hydrogel showed significantly to the inhibition of gram-positive bacteria, *B. subtilis* only.

Authors Contributions

All authors collaboratively contributed to every aspect of the work presented in the manuscript. N. H. S. J. assisted in the laboratory work, analyzed the data and revised the manuscript. A. A. assisted in the laboratory work. D. N. J. was involved in supervision, planning, and editing the paper. S. I. S. S. involved in supervision and provided input and guidelines for the laboratory work. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare that the financial support received from the Ministry of Education Malaysia under grant FRGS19-091-0700 may be perceived as a potential competing interest.

References

Ahmed, A. S., Mandal, U. K., Taher, M., Susanti, D., & Jaffri, J. M. (2018). PVA-PEG physically cross-linked hydrogel film as a wound dressing: experimental design and optimization. *Pharmaceutical Development and Technology*, *23*(8), 751–760. https://doi.org/10.1080/10837450.2017.1295067

Altaf, F., Niazi, M. B. K., Jahan, Z., Ahmad, T., Akram, M. A., safdar, A., Butt, M. S., Noor, T., & Sher, F. (2021). Synthesis and Characterization of PVA/Starch Hydrogel Membranes Incorporating Essential Oils Aimed to be Used in Wound Dressing Applications. *Journal of Polymers and the Environment*, *29*(1), 156–174. https://doi.org/10.1007/s10924-020-01866-w

Alven, S., Nqoro, X., & Aderibigbe, B. A. (2020). Polymer-based materials loaded with curcumin for wound healing applications. In *Polymers* (Vol. 12, Issue 10, pp. 1–25). MDPI AG. https://doi.org/10.3390/polym12102286

Bacha, E. G. (2022). Response Surface Methodology Modeling, Experimental Validation, and Optimization of Acid Hydrolysis Process Parameters for Nanocellulose Extraction. *South African Journal of Chemical Engineering*, *40*, 176–185. https://doi.org/10.1016/j.sajce.2022.03.003

Baghaie, S., Khorasani, M. T., Zarrabi, A., & Moshtaghian, J. (2017). Wound healing properties of PVA/starch/chitosan hydrogel membranes with nano Zinc oxide as antibacterial wound dressing material. *Journal of Biomaterials Science, Polymer Edition*, *28*(18), 2220–2241. https://doi.org/10.1080/09205063.2017.1390383

Bialik-Was, K., Pluta, K., Malina, D., Barczewski, M., Malarz, K., & Mrozek-Wilczkiewicz, A. (2021). The effect of glycerin content in sodium alginate/poly(Vinyl alcohol)-based hydrogels for wound dressing application. *International Journal of Molecular Sciences*, *22*(21). https://doi.org/10.3390/ijms222112022

Butylina, S., Geng, S., Laatikainen, K., & Oksman, K. (2020). Cellulose Nanocomposite Hydrogels: From Formulation to Material Properties. *Frontiers in Chemistry*, *8*. https://doi.org/10.3389/fchem.2020.00655

Butylina, S., Geng, S., & Oksman, K. (2016). Properties of as-prepared and freeze-dried hydrogels made from poly(vinyl alcohol) and cellulose nanocrystals using freeze-thaw technique. *European Polymer Journal*, *81*, 386–396. https://doi.org/10.1016/j.eurpolymj.2016.06.028

Carating, C. A. M., Rosales, R. N. M., Bongao, H. C., & Magdaluyo, E. R. (2019). Polyvinyl alcohol hydrogel reinforcement of cellulose and silica nanoparticles for wound healing application. *Key Engineering Materials*, *821 KEM*, 23–28. https://doi.org/10.4028/www.scientific.net/KEM.821.23

Chen, K., Liu, J., Yang, X., & Zhang, D. (2017). Preparation, optimization and property of PVA-HA/PAA composite hydrogel. *Materials Science and Engineering C*, *78*, 520–529. https://doi.org/10.1016/j.msec.2017.04.117

Cui, L., Tong, W., Zhou, H., Yan, C., Chen, J., & Xiong, D. (2021). PVA-BA/PEG hydrogel with bilayer structure for biomimetic articular cartilage and investigation of its biotribological and mechanical properties. *Journal of Materials Science*, *56*(5), 3935–3946. https://doi.org/10.1007/s10853-020-05467-9

de Lima, G. G., Ferreira, B. D., Matos, M., Pereira, B. L., Nugent, M. J. D., Hansel, F. A., & Magalhães, W. L. E. (2020). Effect of cellulose size-concentration on the structure of polyvinyl alcohol hydrogels. *Carbohydrate Polymers*, *245*. https://doi.org/10.1016/j.carbpol.2020.116612

Jafri, N. H. S. B., Jimat, D. N., Wan Nawawi, W. M. F., Ahmad Nor, Y., & Amid, A. (2024). Optimum Yield of Empty Fruit Bunches Cellulose Nanofibers by Deep Eutectic Solvent and Ultrasonication. *Chemical Engineering & Technology*, *47*(1), 56-67. https://doi.org/10.1002/ceat.202300117

Kamoun, E. A., Loutfy, S. A., Hussein, Y., & Kenawy, E. R. S. (2021). Recent advances in PVA-polysaccharide based hydrogels and electrospun nanofibers in biomedical applications: A review. *International Journal of Biological Macromolecules*, *187*, 755-768. Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2021.08.002

Li, Y., Zhu, C., Fan, D., Fu, R., Ma, P., Duan, Z., Li, X., Lei, H., & Chi, L. (2019). A Bi-Layer PVA/CMC/PEG Hydrogel with Gradually Changing Pore Sizes for Wound Dressing. *Macromolecular Bioscience*, *19*(5). https://doi.org/10.1002/mabi.201800424

Lin, S. P., Lo, K. Y., Tseng, T. N., Liu, J. M., Shih, T. Y., & Cheng, K. C. (2019). Evaluation of PVA/dextran/chitosan hydrogel for wound dressing. *Cellular Polymers*, *38*(1–2), 15–30. https://doi.org/10.1177/0262489319839211

Lv, Q., Wu, M., & Shen, Y. (2019). Enhanced swelling ratio and water retention capacity for novel super-absorbent hydrogel. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *583*. https://doi.org/10.1016/j.colsurfa.2019.123972

Miah, M. A. H., Hasan, M., Sarker, Y. A., Alam, M. M., & Juyena, N. S. (2017). Clinical evaluation of ethanolic extract of curcumin (Curcuma longa) on wound healing in Black Bengal goats. *Journal of Advanced Veterinary and Animal Research*, *4*(2), 181–186. https://doi.org/10.5455/javar.2017.d209

Rahmi, D., Paramadina, S., Anjelika, M., & Widjajanti, R. (2020). Optimized swelling properties of hydrogels based on poly(vinyl alcohol)-carrageenan. *AIP Conference Proceedings*, *2243*. https://doi.org/10.1063/5.0001098

Rezvanian, M., Ahmad, N., Mohd Amin, M. C. I., & Ng, S. F. (2017). Optimization, characterization, and in vitro assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. *International Journal of Biological Macromolecules*, *97*, 131–140. https://doi.org/10.1016/j.ijbiomac.2016.12.079

Rodrigues, F. H. A., Carlos, C. E., Medina, A. L., & Fajardo, A. R. (2019). Hydrogel composites containing nanocellulose as adsorbents for aqueous removal of heavy metals: design, optimization, and application. *Cellulose*, *26*(17), 9119–9133. https://doi.org/10.1007/s10570-019-02736-y

Saleem, M., & Saeed, M. T. (2020). Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent. *Journal of King Saud University - Science*, *32*(1), 805–810. https://doi.org/10.1016/j.jksus.2019.02.013

Shitole, A. A., Raut, P. W., Khandwekar, A., Sharma, N., & Baruah, M. (2019). Design and engineering of polyvinyl alcohol based biomimetic hydrogels for wound healing and repair. *Journal of Polymer Research*, *26*(8). https://doi.org/10.1007/s10965-019-1874-6

Thakur, M., Sharma, A., Ahlawat, V., Bhattacharya, M., & Goswami, S. (2020). Process optimization for the production of cellulose nanocrystals from rice straw derived α-cellulose. *Materials Science for Energy Technologies*, *3*, 328–334. https://doi.org/10.1016/j.mset.2019.12.005

Yang, W., Xu, F., Ma, X., Guo, J., Li, C., Shen, S., Puglia, D., Chen, J., Xu, P., Kenny, J., & Ma, P. (2021). Highly-toughened PVA/nanocellulose hydrogels with anti-oxidative and antibacterial properties triggered by lignin-Ag nanoparticles. *Materials Science and Engineering C*, *129*. https://doi.org/10.1016/j.msec.2021.112385