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The Green Approach in Development of Hydrogel for Wound Healing

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Abstract

Hydrogels derived from plant-based mucilage have potential use in biological materials due to their biocompatibility and high water retention capacity. This study aimed to investigate the effect of the modification of hydrogel with the addition of okra mucilage. Two different methods were used: heated (C2) and non-heated (C1) in the extraction of mucilage, and then, subsequently, characterised in terms of measurements of the swelling ratio, Scanning Electron Microscopy (SEM), and antibacterial activity against *Staphylococcus aureus* (*S. aureus*). It was found that the morphology of the samples demonstrated the porous microstructure, imparting efficient swelling and solvent penetration. Plus, the addition of the heated-extraction okra mucilage enhances both hydration capacity and antibacterial performance in hydrogels with superior swelling capacity of sample C2 (6797%) compared to C1 (5013%), resulting in stronger antibacterial activity. Therefore, the addition of okra mucilage-based hydrogels has potential as wound-healing biomaterials with superior swelling capacity and mechanical strength.

1. Introduction

Hydrogels are three-dimensional crosslinked networks of hydrophilic polymers that incorporate both non-covalent interactions, including hydrogen bonds, ionic, and covalent interactions. This unique structural configuration confers remarkable water uptake capacity, high porosity, flexibility, and softness, making them useful in diverse biomedicine and environmental applications [1],[2]. Owing to their inherent biocompatibility and tunable physicochemical properties, hydrogels have been extensively researched for controlled drug release, tissue engineering scaffolds, and wound management systems. Not only that, they also function in environmental remediation by acting as absorbents and immobilizing pollutants [1],[2].

Natural polysaccharides, which are typical hydrogel-forming agents because of their biocompatibility, biodegradability, and capacity to hold water [3],[4]. However, their clinical translation is usually constrained by limited aqueous solubility and reduction of bioavailability of okra and other botanical-derived polymers [5], [6].

Okra (*Abelmoschus esculentus*) is a widely cultivated vegetable known for its high nutritive value and abundance of bioactive phytochemicals, including vitamins, minerals, anti-diabetic agents, and bioactive compounds, with wound-healing potential [3],[7],[8]. Thus, the extraction of Okra gum polysaccharides derived from okra stem exhibits hydrophilic behaviour, imparted to the synthesis of a hydrogel, which is applicable in enhancing drug delivery and wound healing due to its hydrophilic properties.

Cotton fabric-based substrates, frequently used in wound dressings, are well-known for their biocompatibility, biodegradability, and high moisture sorption capacity, as well as due to intermolecular hydrogen bonding within the cellulose matrix [9]. Some previous studies on the incorporation of microcrystalline cellulose (MCC) based hydrogel with cotton fabric have demonstrated efficacy in wound

management from medium-to-heavy exudate. Proper wound care included preserving an environment that promoted healing while protecting against further damage and infection [7], [10].

Conventional dressings like gauze and cotton wool have the potential to adhere to wound surfaces and promote secondary tissue damage upon removal, thereby impeding re-epithelialization [11],[12]. Previous study on the neem gum-based hydrogels with encapsulated antibiotics showed promise in wound healing, though there are limitations in oxygen diffusion [13]. This current study investigates the utilization of okra mucilage as a structural and functional polysaccharide for the modification of hydrogels in order to improve the mechanical structure, retain moisture, and provide essential nutrients for cell growth.

2. Material and Methods

2.1 Materials

In this study, fresh okra (*Abelmoschus esculentus*) pods were purchased from the neighborhood farmer's market. The chemicals, such as guar gum (GG), acrylamide (AAm), and ethanol, were purchased from the Chemiz brand. Meanwhile, Polyethylene terephthalate (PET) textile was purchased from Shuzou Origin Environmental Protection Technology Co., Ltd., and citric acid (CA) from Sigma-Aldrich.

2.2 Methods

2.2.1 Extraction of Okra Mucilage from Okra Pods

Fresh okra pods were thoroughly cleaned, sliced, and then soaked in water for 12 hours. After that, the soaking mixture was filtered through muslin fabric to extract the mucilage and subsequently precipitated from the aqueous phase using ethanol at a 1:3 v/v ratio. The resulting mucilage was then milled into a fine powder and left to dry at room temperature for an entire day. Alternatively, the extraction of okra was executed in two different methods: without heat (at room temperature) and with the addition of heat (temperature, 70°C), which were labelled as C1 and C2, respectively. Again, thinly sliced fresh okra was heated for 3 hours as part of a hot extraction process and then extracted and precipitated in the ethanol. Final samples of the mucilage were then baked at 60°C for 3 hours to dry them out before grounding them into fine powder.

2.2.2 Pre-treatment of Polyethylene Terephthalate (PET) Textiles

Polyethylene Terephthalate (PET) fabrics were first immersed in a 1.0 M NaOH solution at 60℃ for 2 hours. The temperature was checked every 15 minutes to ensure that the temperature was not over 60°C. Next, the PET was taken out using tweezers and was submerged in ethanol and then washed with distilled water twice before being placed on a plastic petri dish. This procedure had been repeated for the remaining PETs but used the new distilled water for each PET. Lastly, all the petri dishes were dried in a drying oven for a day.

2.2.3 PET Dip Coating

About 5 ml of extracted pre-gel solutions of okra mucilage (OM) were synthesized by stirring the solution for 10 minutes to ensure homogenous dispersion. Then, coating polymerization occurred when the PET textile was immersed in the petri plates containing the pre-gel solution. After samples were dried in the oven at 60℃ for 1 hour to evaporate the solvent and aid in the hydrogel's adhesion to the PET surface.

2.3 Characterization of Okra Mucilage

2.3.1 Swelling Ratio of Dried Okra Mucilage Sample

The swelling ratio of Okra Mucilage, represented in Table 1, was calculated based on Equation (1) presented below.

$$\frac{W_f}{W_i} \times 100\% \tag{1}$$

where:

W_f = Final weight of the okra sample after soaking in water for 24 hours.

W_i = Initial weight of the okra sample before soaking in water for 24 hours.

Equation (1) was used to calculate the swelling ratio of the dried okra sample after soaking in water for 24 hours. This is crucial to determine the water absorption capacity of the okra powder, which directly affects the hydrogel's ability to maintain a moist environment conducive to wound healing.

2.3.2 Morphology via Scanning Electron Microscope (SEM)

The morphologies of okra mucilage samples (C1 and C2) were analyzed via a scanning electron microscope (SEM) at a voltage of 15 kV. To enhance surface conductivity and reduce charge effects, an ion sputter coater was used to impart a gold coating to the samples before the analysis. The coating is purposely designed to lead to clear and high-resolution imaging of the materials' surface morphology and structure. The morphologies of the sample were taken at 200 and 2000x magnifications.

2.4 Characteristic of Hydrogel Patches

The grafting effect of the hydrogel patch samples was determined by using a high-accuracy digital calliper to measure their thickness. This technique allowed for a thorough assessment of the thickness differences brought on by various okra mucilage concentrations (C1 and C2). An initial thickness of the treated and untreated patches was measured in order to quantify the incremental thickness variations brought about by the addition of *okra mucilage*. This method makes it easier to expand the thickness of the hydrogel patches, which is important for their possible use in wound-healing and biomedical applications.

2.5 Antimicrobial Activity of Okra Mucilage

The antibacterial activity of okra mucilage was investigated against *Staphylococcus aureus* (*S. aureus*) in the inhibitory zone. A *S. aureus* colony was streaked over nutritional agar in each of the four quadrants by using a blunt wooden stick. The colony was then incubated for 16 hours at 37°C. After incubation, colonies were checked for development. A single colony was selected and inoculated into 20 mm of broth using a wooden stick. The incubation was kept at 37°C with 30 rpm agitation for 16–18 hours. Bacterial dilution was done with a spectrophotometer to obtain an optical density (OD) range of 0.08–0.12 at 625 nm. All equipment and supplies, including agar and nutritious broth, were autoclaved before use. Nutrient agar was added to Petri dishes, which were then allowed to dry. Bacteria were added to the agar using microbial swabs, resulting in five holes per plate at the specified C1 and C2 concentrations (1%, 5%, and 7%). There is a label for each hole. Agar was added to each hole using a micropipette, and it was allowed to dry. The holes that had been marked were then filled with bacterial samples that had been diluted with nutritional broth. Amoxicillin was used to treat the positive control holes, while autoclaved distilled water was used to fill the negative control holes. To ensure that the samples in the micropipettes did not spill, close supervision was maintained, and each pipette tip, microbiological swab, and toothpick used for puncturing holes in the plate was used only once.

3. Results and Discussions

3.1 Swelling Ratio

The swelling ratio of dried okra mucilage was calculated using Equation (1), which divides the okra sample's ultimate weight after soaking in water for 24 hours by its starting weight prior to soaking. Determining the okra powder's water absorption capacity was essential to assessing its potential for use in hydrogel formulations for wound patches. A moist environment is necessary for effective wound healing, and this is primarily based on the hydrogel's ability to absorb water and swell. Table 1 shows the swelling ratio of dried okra mucilage C1 and C2 samples.

Table 1 *Swelling Ratios of Dried Okra Mucilage C1 and C2 samples after soaking in water for 24 hours*

Sample Type	C1	C2
Initial Weight (g)	0.088	0.013
	0.022	0.014
	0.009	0.015
Final Weight (g)	4.431	1.119
	0.563	1.097
	0.691	0.639
Average Increase (%)	4929	6698

It is clearly seen that the C2 sample (6698%) is significantly higher compared to the C1 sample (4929%). The plausible reason is that the C2 sample demonstrated an increment in water absorbency affected by the

heating process, whereas heat-induced modification has caused the modification of its molecular structure, making it more hydrophilic and prone to swelling. Therefore, the C2 sample exhibits better compatibility in hydrogel coating for wound dressings. The swelling ratio is a crucial parameter for hydrogels used in wound dressings because it influences the hydrogels' ability to retain moisture in the wound and absorb exudates, both of which aid in the healing process. Hydrogels with a higher swelling ratio are superior in managing wound exudates, reducing the risk of infection, and promoting faster healing. It was observed that these samples maintain a moist environment, which encourages cell migration and multiplication, hastening the healing process [11],[14], [15]. A previous study found that a higher swelling ratio of the heated okra mucilage (C2) suggests that it could be a great starting point to produce hydrogels meant for use in wound care applications [16].

3.2 Scanning Electron Microscope (SEM)

The surface morphology of okra mucilage powders on different extractions (with and without heat treatments) was photographed in SEM images, as seen in Fig. 1 (a-d). These SEM images indicated the change in surface morphology of okra powder affected by heating. More uneven and rough surface with micro-agglomerated particles formation in the C2 sample compared to the C1 sample, as can be seen in Fig. 1 (a) and Fig. 1 (b). The regular structural morphology was interrupted with a heterogeneous surface in the SEM image of the C2 sample. After coating with textile, the attachment of the extracted okra sample is clearly seen in Fig. 1 (c) and Fig. 1 (d), which shows an agglomeration and porous structures of extracted okra onto the modified C1 and C2 hydrogel patch surface.

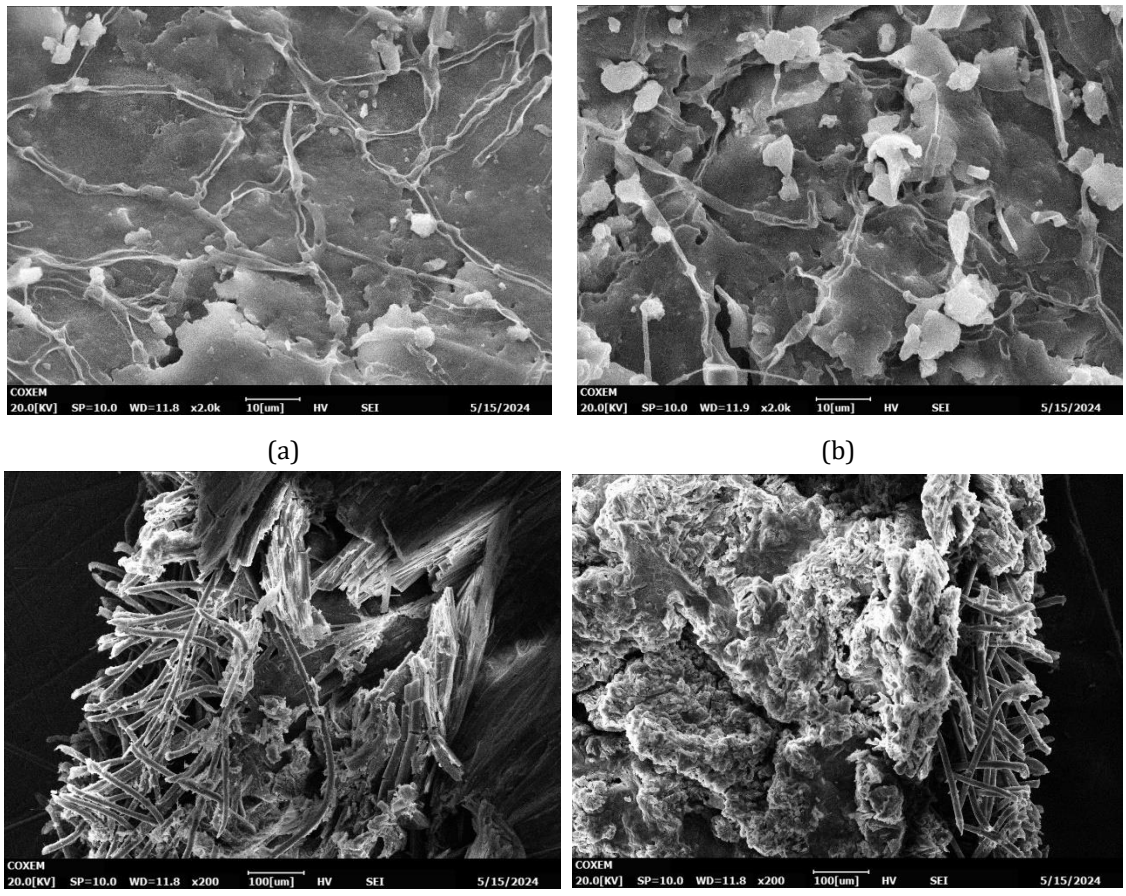

Fig. 1 *The morphologies of sample C1 and C2 sample SEM analysis of okra mucilage powders:(a) C1 sample powder (no-heat treatment) okra powder; (b) SEM micrograph of C2 (heat treatment) okra powder; hydrogel patch in cross section area; (c) C1 sample (no-heat treatment) patch; (d) C2 sample (heat treatment) patch*

3.3 Characteristic of Hydrogel Patches

The thickness of the modified hydrogel patches was taken to investigate the effect of coating on the formation of the modified okra-hydrogel patch. The thickness of the hydrogel patches after the coating process was measured at different concentrations, as shown in Table 2.

Table 2 *Thickness measurements of okra-based hydrogel patches at various concentrations*

Sample	Concentration (%)	Thickness (mm)
Untreated patch	N/A	0.20
Treated patch	N/A	0.20
C1	5	0.21
C2	5	0.21
	10	0.22
	15	0.23
	20	0.21

From Table 2, the treated textile patch demonstrated a remaining thickness similar to that of the untreated one. Meanwhile, the thickness increases somewhat to 0.21 mm for the C1 and C2 samples at a 5% concentration, suggesting that the hydrogel patch's overall thickness may increase somewhat even at low okra mucilage concentrations. This indicates alkaline conditions do not significantly affect the textile patch surface but only promote a slight activation of functional groups on the surface that led to the formation of hydrogel patches. As the amount of okra mucilage in the C2 samples increases, a further increase in thickness is shown; at a 15% concentration, this thickness can approach 0.23 mm. This increase is caused by the higher mucilage content in okra, which presumably makes the gelation and cross-linking processes easier, resulting in a denser and slightly thicker hydrogel matrix. However, at a 20% concentration, the thickness returns to 0.21 mm, indicating that the reason additional mucilage does not further increase thickness above a specific concentration could be due to saturation effects or limitations on matrix formation.

These results suggest that the concentration of okra mucilage has a considerable but non-linear influence on the thickness of the hydrogel patches. The first increase in thickness with increased mucilage concentrations illustrates the effective integration and swelling features of okra mucilage in the hydrogel matrix. However, the drop at the greatest dosage tested would imply that there is a range of concentrations that work well to get the right thickness without going overboard. This is significant because it strikes a balance between the hydrogel wound dressings' mechanical properties and effectiveness.

3.4 Antimicrobial Effect of Okra Mucilage

Table 3 shows the antimicrobial activity of okra mucilage, expressed as *dead zone* (µm), was measured for two sample types (C1 sample: unheated; and C2 sample: heat-treated) at different concentrations of 1%, 5% and 7%. Overall, the results show that higher mucilage concentrations generally correspond to larger dead zones. Indicate stronger antibacterial effects.

Table 3 *Antimicrobial activity (dead zone) of okra mucilage for C1 and C2 samples at different concentrations*

Sample Type	Conc. (%)	Dead Zone (µm)			Average
		Replicate 1	Replicate 2	Replicate 3	
C1	1	416.67	521.23	583.33	507.08
	5	724.62	641.63	745.76	704.01
	7	914.85	617.17	655.24	729.09
C2	1	514.95	583.34	625.09	574.46
	5	837.52	790.08	573.93	733.84
	7	856.23	725.20	708.33	763.25

For C1, the average dead zone increased from 507.08 µm at 1% to 704.01 µm at 5%, producing an average increase of approximately 39%, while the increase from 5% to 7% (729.09µm) only increased about 3.6%. Similarly, C2 showed a corresponding increase of approximately 27.8% from 1% (574.46µm) to 5% (733.84µm), but only about 4% from 5% to 7% (763.25 µm). These trends suggest that the most significant improvement in antibacterial activity occurs between 1% and 5%, with diminishing returns at higher concentrations. In these applications, antimicrobial properties are essential for preventing infection, maintaining biocompatibility, and

ensuring long-term stability. Hydrogel with inherent antimicrobial activity is more effective in infection control, remains functional for longer periods, and provides enhanced patient safety [17].

4. Conclusion

It can be seen that a detailed analysis of hydrogels based on okra mucilage offers significant new insights into potential biological applications. The swelling ratio tests demonstrated that heat treatment significantly increased C2 sample capacity to absorb water in comparison to C1, which is necessary for hydrogel performance in wound healing applications. These findings were proven by SEM images, where both the heat-treated and untreated samples have porous and rough surface features that are necessary for solvent penetration and swelling efficiency. The nonlinear relationship between okra mucilage concentration and hydrogel thickness highlights the need for an optimal formulation that balances mechanical strength and swelling capacity. The antimicrobial test results demonstrated that increased antibacterial activity is shown by broader dead zones, which are linked to higher okra mucilage concentrations. This was particularly noticeable in sample C2, where the antibacterial properties might have been enhanced by heat treatment. Thus, these modified hydrogels based on okra mucilage have promise as practical biomaterials for wound dressings.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Franceona Fiona Perancis, Muhammad Akmal Md Hanif, Mohd Fhakrur Radzi Mohd Fhadlee, Siti Samahani Suradi;* ***data collection*** *Franceona Fiona Perancis, Muhammad Akmal Md Hanif, Mohd Fhakrur Radzi Mohd Fhadlee, Siti Samahani Suradi;* ***analysis and interpretation of results:*** *Franceona Fiona Perancis, Muhammad Akmal Md Hanif, Mohd Fhakrur Radzi Mohd Fhadlee, Siti Samahani Suradi;* ***draft manuscript preparation:*** *Franceona Fiona Perancis, Muhammad Akmal Md Hanif, Mohd Fhakrur Radzi Mohd Fhadlee, Siti Samahani Suradi; All authors reviewed the results and approved the final version of the manuscript.*

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