Hindawi
International Journal of Polymer Science
Volume 2019, Article ID 7651810, 11 pages
https://doi.org/10.1155/2019/7651810

Research Article

Fabrication of an Original Transparent PVA/Gelatin Hydrogel: *In Vitro* Antimicrobial Activity against Skin Pathogens

Naila Imtiaz,[1,8] Muhammad Bilal Khan Niazi,[2] Fehmida Fasim,[3] Barkat Ali Khan,[4] Syeda Asma Bano,[5] Ghulam Mujtaba Shah,[6] Malik Badshah,[7] Farid Menaa,[8] and Bushra Uzair[1]

[1]*Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan*
[2]*Department of Chemical Engineering, National University of Sciences & Technology, Islamabad, Pakistan*
[3]*Discipline of Biomedical Science, Sydney Medical School, The University of Sydney, Sydney, NSW, Australia*
[4]*Department of Pharmaceutics, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan*
[5]*Department of Microbiology, University of Haripur, Haripur, Pakistan*
[6]*Department of Microbiology, Hazara University, Mansehra, Pakistan*
[7]*Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[8]*Department of Advanced Technologies, California Innovations Corporation, San Diego, CA, USA*

Correspondence should be addressed to Bushra Uzair; bushra.uzair@iiu.edu.pk

Received 11 October 2018; Revised 20 December 2018; Accepted 31 December 2018; Published 14 March 2019

Guest Editor: Mingqiang Li

The design of actively efficient and low-toxicity formulations against virulent bacterial strains causing skin infections remains a challenging task. The aim of the present study was to develop and evaluate *in vitro* a hydrogel impregnated with a known plant extract for topical applications against major skin bacteria. A poly(vinyl alcohol) (PVA)/gelatin hydrogel, namely HG, was prepared by esterification following the solution casting method. The gelling process was realized by cross-linking the synthetic polymer PVA and the biopolymer gelatin in the presence of hydrochloric acid (HCl). A crude extract of *Nigella sativa* seeds was then encapsulated in HG, and the resulting HGE was characterized morphologically (by Scanning Electron Microscopy (SEM)), structurally (by X-ray powder diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy), behaviorally (by swelling behavior), and biologically (by the agar well diffusion method). The results of HGE were compared to HG and HG impregnated with 10% acetic acid (HGAA). SEM sections of HGE revealed a dense and porous surface, suggesting a good hydrophilicity. X-ray diffractograms indicated that HGE and HG had a similar degree of crystallinity. FTIR spectra confirmed that esterification occurred between PVA and gelatin suggesting that the amine group is involved in the intercalation of the plant extract components in HG. Further, HGE was found to be as wettable and swellable as HG, suggesting a good biocompatibility. Eventually, HGE exerted a pronounced inhibitory effect against two major skin pathogens, the Gram-negative *Pseudomonas aeruginosa* and the Gram-positive *Staphylococcus aureus*, suggesting a good extract release. Taken together, the experimental data indicated that HGE might be a promising wound dressing.

1. Introduction

Despite advancements in the standards of healthcare and medical technology (i.e., sterilization and aseptic techniques), diseases caused by pathogens (e.g., bacteria, viruses, parasites, or fungi) remain major public health threats leading to struggling socioeconomic issues. Indeed, according to a World Health Organization (WHO) report, infectious diseases represent the second leading cause of mortality worldwide [1]. The increasing prevalence of infections, especially those associated with impaired wound healing and biomedical implant or device (e.g., catheter) failure [2], has spurred the development of new polymer formulations capable of exerting an antimicrobial activity [3].

S. aureus and *P. aeruginosa* represent the most common pathogens isolated from both acute and chronic injuries of different etiologies (e.g., wounds and burns), and so the current research is mainly focused in controlling their spread to avoid sepsis [4].Interestingly, wound and burn healing occurs more quickly with the help of dressing biopolymers such as hydrogels [4]. Hydrogels represent a class of highly hydrated 3D materials made of synthetic and natural polymers (e.g., polysaccharides such as alginate, starch, dextran, chitosan, or their derivatives, proteins such as gelatin and fibrin, polypeptides, and polynucleotides) [5, 6]. Their valuable properties, including hydrophilicity, biocompatibility, biodegradability, flexibility, and other mechanical properties similar to natural tissues, represent a tremendous interest in medicine. Thereby, their end use is quite noticeable in the tissue engineering (e.g., healing burns and wound dressing/wound fillers, contact lenses, absorbable sutures, hybrid-type organs such as encapsulated living cells, prostheses, and coated implants), pharmaceutical (e.g., drug delivery), and biomedical fields (e.g., asthma and osteoporosis treatments) [7].

Since the last decade, much attention was gained in the preparation and characterization of hydrogels, which are considered as a starting point when engineering antimicrobial materials [5]. Indeed, in addition to their possible inherent antimicrobial activities, particularly against multidrug resistant strains [8], they can be designed to convey acting antimicrobial agents that can be locally released over time, either through (noncovalent) encapsulation and/or (covalent) immobilization/coating [9]. Recently, Marchesan et al. described a relatively safe antimicrobial hydrogel that is formed *via* the self-assembly of the hydrophobic tripeptide ((D) Leu-Phe-Phe), which in the presence of ciprofloxacin took an active part in the integration of the drug into the peptide network allowing its eventual controlled release [10]. Nowadays, hydrogels made of PVA cross-linked with natural polymers (e.g., gelatin and polysaccharides) are well-studied for their biomedical applications and have long been particularly designed as wound dressing materials [11]. Interestingly, hydrogel features, including behavioral (e.g., swelling) and mechanical (e.g., strength) properties, may be modulated/optimized by the quantity of a given cross-linking agent or by the number of repeated freezing and thawing (F-T) cycles [12]. To date, there is still a paucity of reports on hydrogels impregnated with a plant extract exerting an antimicrobial activity. *N. sativa* L. (aka, black cumin) is largely considered as a marvelous herb that can cure numerous infirmities [13] and infections [14]. Nonetheless, the encapsulation of antimicrobial extracts from *N. sativa* has not been explored in hydrogels yet.

In this original *in vitro* study, we prepared and characterized HGE for its possible use as a scaffold for faster wound recovering/healing or for burn or skin infection management. The preparation was done according to a previously optimized method [15] using the casting method and a chemical cross-linker easily removable by washings. The morphology, structure, behavior, and potential antibacterial activity of HGE against the major skin pathogens, *S. aureus* and *P. aeruginosa*, were assessed.

2. Material and Methods

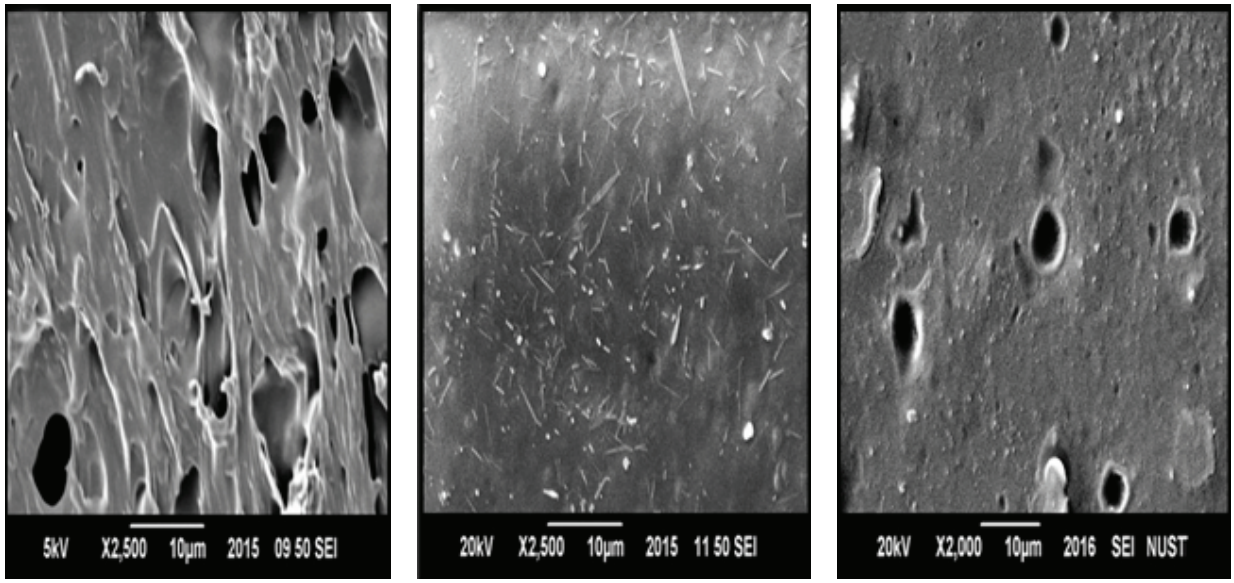
2.1. Chemicals, Strains, and Apparatus. The following commercially available chemicals were purchased from the indicated manufacturer and applied throughout this work without further purification. PVA (molecular weight: 125,000), HCl (35% pure), acetic acid, and Type B gelatin (~225 Bloom, for bacteriological purposes) were all purchased from Sigma-Aldrich Corp., St. Louis, MO, USA. Ethyl acetate was purchased from BDH Laboratory Supplies, England. Ethanol was purchased from Merck KGaA, Darmstadt, Germany.

Dry seeds of *N. sativa* were purchased locally and converted to powder form. Microbiological Oxoid™ culture media (i.e., Mueller-Hinton broth (MHB) and tryptone soy agar (TSA)) were supplied by Thermo Fisher Scientific Inc. *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 9144) were obtained from the American Type Culture Collection (ATCC), Manassas, VA 20108, USA. Oxoid™ Vancomycin (# CT0058B) and Gentamycin (# CT0024B) antimicrobial susceptibility discs were purchased from Thermo Fisher Scientific Inc.

The thermostatic incubator DHP-9052, the high-speed refrigerated centrifuge TGL20MC, and the multifrequency ultrasonic cleaning machine NB-600 DTY were all purchased from Zhengzhou Nanbei Instrument Equipment Co. Ltd., China. The X-ray diffractometer IPDS II was purchased from STOE & Cie GmbH, Darmstadt, Germany. The SEM Quanta 450 FEG was purchased from Thermo Fisher Scientific Inc. The FT/IR-4100 type A spectroscope was purchased from JASCO Inc., Easton, MD, USA.

2.2. Preparation of N. sativa Crude Extract. 2 grams of *N. sativa* seeds were crushed to granules using a stone rotor and poured into a beaker. Then, following a routine protocol [16], 8 mL of ethyl acetate (10%) was added to the beaker and the extract (1 : 4 w/v) was kept at room temperature for three days. The beaker remained uncovered to evaporate the maximum amount of solvent. The resulting concentrated extract was stored in a sterile container in a refrigerator until further use.

2.3. Preparation of Hydrogels. PVA/gelatin hydrogels were prepared with a weight composition of 75/25 under sterile conditions using the method described by Uzair et al. [14], with little modification. Briefly, 10 g of PVA was added into 100 mL of DD water (10% w/v), which was heated at 100°C for 1 hour under constant mechanical stirring (3500 rpm) to obtain total dissolution. The resulting homogeneous transparent aqueous solution was then mixed with 2.5 mg of gelatin and 0.05 mL of HCL (35%). The subsequent dispersion was stirred at 70°C for 30-45 minutes under constant mechanical stirring (100 ± 5 rpm) to carry out the esterification reaction between PVA and gelatin. Eventually, 100 mL of the thick dispersion solution was subdivided into three equal parts (33.3 mL each). Solution 1, in which nothing was added, served as the "internal control." Solution 2, in which 5 mL of acetic acid (10%) was added, served as the "external control." Solution 3, in which



(a) (b) (c)

Figure 1: SEM micrographs representing the topographic view of the prepared hydrogels: (a) HG; (b) HGAA; (c) HGE.

5 mL (equivalent to 1 g) of the crude extract of *N. sativa* was added, served as "test."

These three solutions were separately mixed and exposed to ultrasonic waves for 2 hours in order to homogenize and remove bubbles/air, respectively. Then, each solution was converted into a membrane by the conventional solution casting method. Thereby, the three solutions were poured independently in standard 90 mm petri dishes and left overnight for drying at room temperature. Importantly, they were washed thoroughly with DD water to remove HCl and subsequently dried at 37°C for 3 hours in an oven. Then, 2 mL of glycerol was added to overcome the brittleness of the membrane. Eventually, the prepared hydrogels, i.e., HG (PVA/gelatin), HGAA (HG+acetic acid), and HGE (HG+extract), were peeled off with the help of forceps and stored in airtight pouches/ziplock packets until further use.

2.4. Characterizations of the Prepared Hydrogels

2.4.1. Scanning Electron Microscopy (SEM). To obtain high-resolution imaging of surface morphology, all the sterile hydrogels were subjected to SEM measurements using 1/1 cm samples coated with gold. This coating is required to obtain a clear image/micrograph of an insulating material, although it is so thin (200 Å) that it does not hinder the identification of specific minerals [17].

2.4.2. X-Ray Powder Diffraction (XRD). In order to determine the crystallinity of all the hydrogels, XRD (STOE & Cie GmbH, Darmstadt, Germany, θ-θ) was performed using Cu radiation generated at 40 kV and 40 mA. The range of the diffraction angle was 10 to 70° 2θ. The 0.02° step size of 2θ was maintained at a scan speed of 2 s/step. PVA 10% was used as a supplementary control.

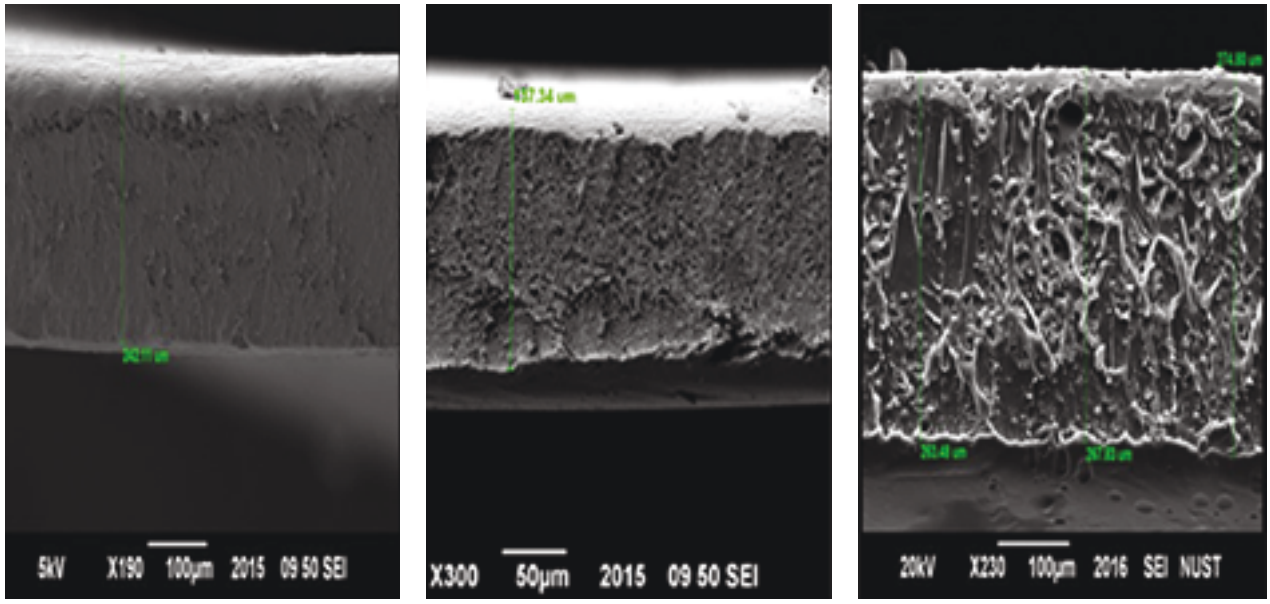
2.4.3. Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectroscopy was employed to characterize the presence of specific functional chemical groups and their interactions/complexations in HG and HGE. The cross-linking of PVA with gelatin was also checked with this technique [10]. The prepared hydrogels were milled and mixed at a ratio of 1.0% to KBr powder dried for 24 hours at 120°C. FTIR spectra were obtained in the range of 4000-400 cm^{-1}, with a scanning speed of 2 mm/sec and a 4 cm^{-1} resolution.

2.4.4. Swelling Behavior. The dynamic swelling behavior of HG and HGE was investigated to determine the mechanism of water transport through these hydrogels. Thereby, the hydrogels were cut and the initial weight was determined (W_i). The samples were then kept in DD water (swelling medium) at room temperature. Subsequently, the samples were taken out from the DD water (pH~5.6) at regular intervals of time (i.e., every 2 hours for 8 hours), and each sample weight was determined at room temperature (W_f). Special care was taken to ensure that there was no water present during weighting. The process was continued until the saturation of final weight is observed. Swelling percentage was calculated using the following formula [18]:

$$\text{Swelling\%} = \left[\frac{W_f - W_i}{W_i}\right] \times 100, \quad (1)$$

where W_f is the weight of the product after hydration, and W_i is the weight of the dried product.

2.4.5. Antimicrobial Activity. Skin pathogens, i.e., *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were cultured aerobically in Mueller-Hinton broth (MHB) and subcultured on tryptone soy agar (TSA), as reported by another group [19]. The antimicrobial potential of the prepared gel was tested



(a) (b) (c)

Figure 2: SEM micrographs representing the transverse view of the prepared hydrogels: (a) HG; (b) HGAA; (c) HGE.

by the agar well diffusion method described elsewhere [20], with little modification. Inoculums of *S. aureus* (ATCC 9144, the so-called "Oxford *Staphylococcus*") [21] or *P. aeruginosa* (ATCC 27853) were prepared from a 24-hour culture on *Luria-Bertani* (LB) broth, and the turbidity of the suspension made in a sterile saline solution (0.85%) was adjusted with a spectrophotometer at 530 nm to obtain a final concentration that matches a 0.5 McFarland standard ($0.5\text{-}2.5 \times 10^3$). 20 mL of Mueller-Hinton *agar* (*MHA*) was melted, cooled to 55°C, and poured into a 90 mm petri dish. The plates were allowed to cool down on a leveled surface. Once the medium had solidified, each bacterial strain was swabbed uniformly across a MHA plate. Then, 6 mm wells were cut out of the agar, and hydrogels (i.e., HG, HGAA, and HGE) cut in 6 mm diameter pieces were placed into each of these wells. Control antibiotic discs, i.e., Gentamycin (10 μg) for *S. aureus* or Vancomycin (30 μg) for *P. aeruginosa*, were placed on the respective plates. Eventually, the inoculated plates were incubated at 37°C for 24 hours, after which the respective sizes of the zones of inhibition were measured (in mm). CLSI disc diffusion breakpoints were used for the interpretation of the zones of inhibition against the control antibiotic discs [22].

2.5. Statistics. The statistical analysis of most results (i.e., SEM, swelling behavior, and antimicrobial assays) was performed using the software package SPSS Statistics 17.0 following one-way analysis of variance (ANOVA), at a significance level of 95%. ANOVA statistical analysis was used to determine whether the difference between the groups studied was significant. To ensure accuracy, all data were obtained from three sets of independent experiments. Data were presented as means ± standard error of the mean (SEM) with statistical annotations when required. Differences with $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Microarchitecture of the Synthesized Hydrogels. Topographic view (i.e., surface) and transverse view (i.e., thickness) of HG, HGAA, and HGE were obtained by SEM (Figures 1 and 2). The topographic microscopic view of HG (Figure 1(a)) revealed a complex surface, i.e., a crispy 3D inner structure containing irregular voids arranged within disorganized/nondirectional lamellas, suggesting valuable hydrophilic characteristics. However, the topographic view of HGAA (Figure 1) indicated a unique crystallized surface, exempt of voids, which may be due to the partial dissolution of acetic acid in the final solution. Interestingly, the topographic analysis of HGE (Figure 1) depicted a less complex microarchitecture than HG, i.e., a granular, rough, and porous surface containing a few aerosols with clearer delimited voids, which is representative of a good hydrophilicity. Besides, we noticed that the expected impregnation of the extract did not appear to have a significant effect on membrane structure distortion. The preparation, properties, and applications of such type of hydrogel have been previously described [15]. SEM analyses of HGE reveal a dense and porous-like inner structure with a less complex microarchitecture than HG, although the size and distribution of macropores were not completely investigated by porosimetry. These data are usually indicative of a good hydrophilicity, a natural characteristic of hydrogels [23]. The SEM images of HG also evolve a dense membrane and evidence a typical 3D structure, the data of which are consistent with a previous study [24].

The transverse microscopic view of HG (Figure 2) revealed a significantly ($p < 0.05$) higher average thickness ($241.49 \pm 0.55\,\mu$m) than HGAA (Figure 3(b)) ($138.37 \pm 0.51\,\mu$m). Interestingly, the average thickness of HGE ($270.32 \pm 1.54\,\mu$m) was significantly ($p < 0.05$) the highest (Figure 2), and it could be explained by a higher

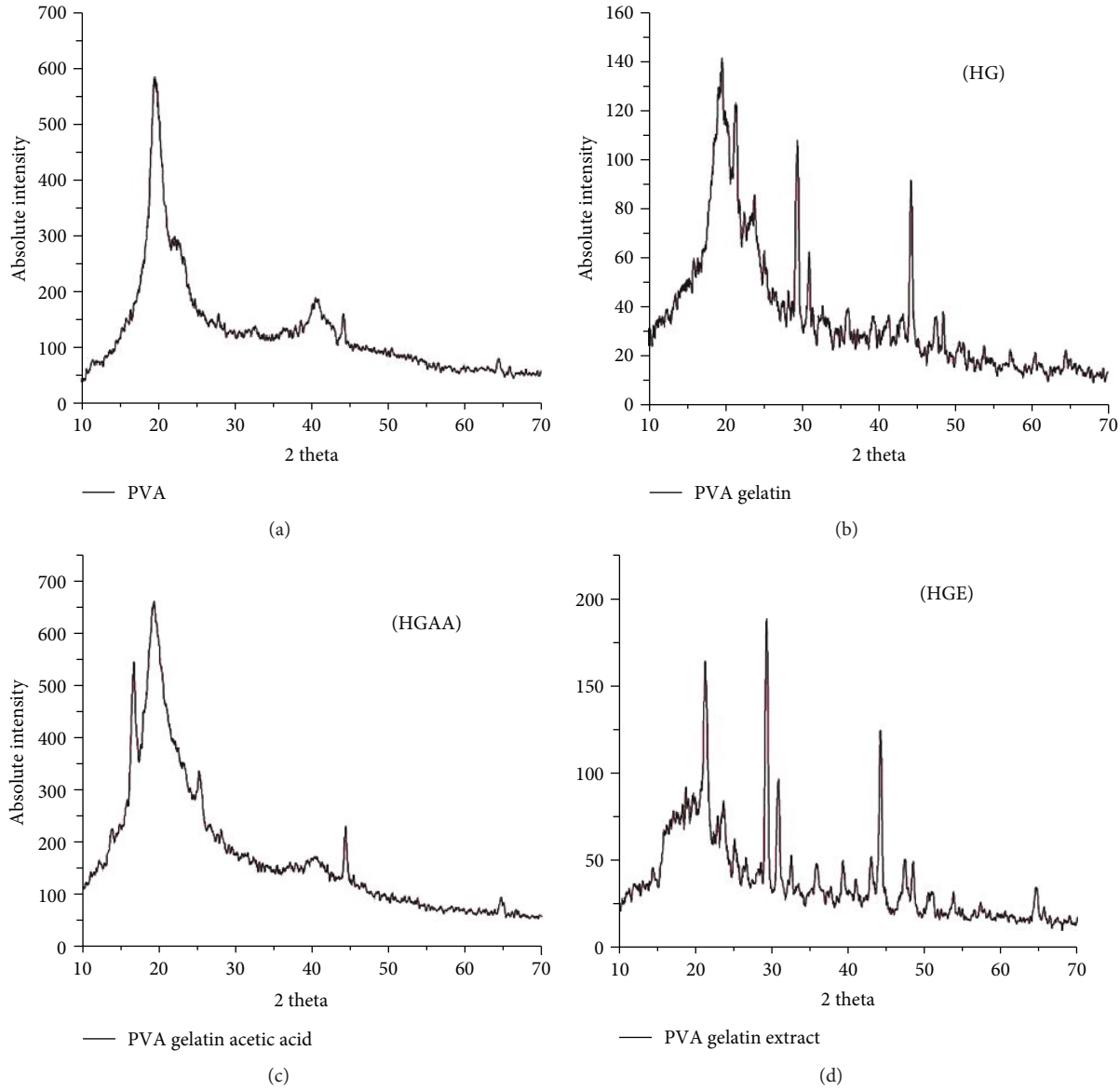


Figure 3: X-ray diffractograms of the prepared hydrogels: (a) PVA; (b) HG; (c) HGAA; (d) HGE.

viscosity, an important parameter for the hydrogel composition. Nie et al. [11] reported that composite or hybrid hydrogels (e.g., PVA/gelatin hydrogel) are the best choice of material compared to other dressing forms because they fit the requirements for the ideal wound dressing, including better mechanical stability at the swollen state (its intrinsic properties include biocompatibility, hydrophilicity/high degree of swelling in aqueous solution, elasticity, resemblance to rubber, and absence of detectable toxicity) [3]. Besides, gelatin is a natural polymer that is able to activate macrophages, displays a high hemostatic effect, and increases the swelling % capacity of a PVA hydrogel, making it very useful for a wide variety of wound dressings [25]. The possible reason why the topographic view of HG and HE showed a significant difference may be because the system turned acidic in the gel with acetic acid, and there is a possibility that the nature of the gelation mechanism was changed.

Table 1: Absolute intensities of the main peaks at 2θ from XRD patterns of the prepared hydrogels.

Hydrogels types	20° 2θ	28° 2θ	40° 2θ	44° 2θ
PVA	582	ND*	186	160
HG	142	108	36	188
HGAA	665	340	175	234
HGE	164	92	50	124

Nie et al. [11] reported that the in acidic system, the nature of the gelation mechanism is deprotonation and entanglement. The difference in the gelation mechanism leads to a great difference in the gelation process, hence the topography of different gels was changed. The HE cross-linked mesh-like structure, with interconnected micropores evident in Figure 2(c), provides efficient channels for rapid water

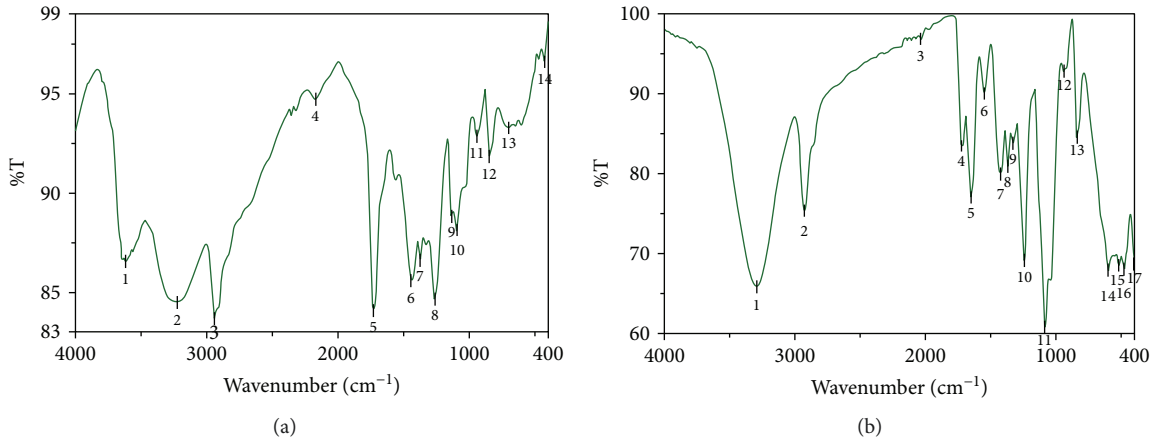


Figure 4: FTIR spectra of the prepared hydrogels: (a) HG; (b) HGE.

Table 2: Main FTIR spectral assignments in the prepared hydrogels.

Hydrogel	Wave number (cm^{-1})	Peak intensity	Nature of bond(s)	Spectral assignment #
HG	3615	Low	O—H	1
	3222	Low	H—H	2
	1730	Low	C=O	5
	1443	Medium	C—H	7
HE	3291	Low	—NH_3^+	1
	1652	Strong	C=O and NH_2	6
	1087	Low	C—O	11
	943	Strong	CH_2	12

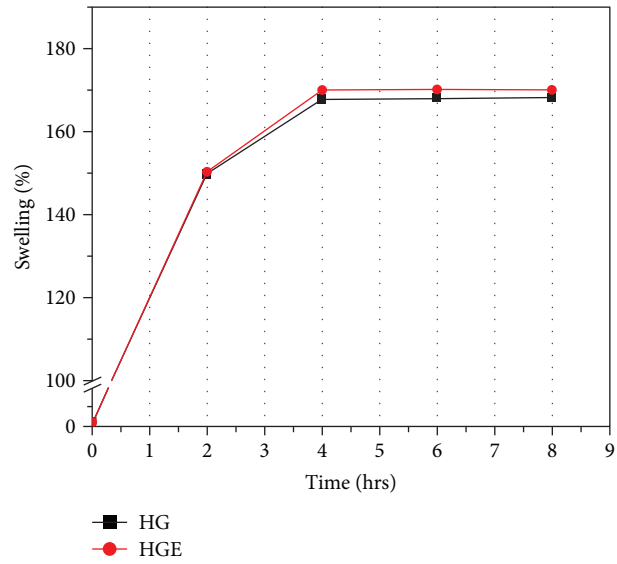


Figure 5: Swelling behavior of the prepared hydrogels over a period of 8 hours.

transport, which explains the potential of our hydrogel samples reaching equilibrium swelling in only 8 hours. In the acidic system, the gelation process is promoted by the diffusion of OH^- from the coagulation bath instead of the formation of cross-links in the whole system as observed in Figure 1(b).

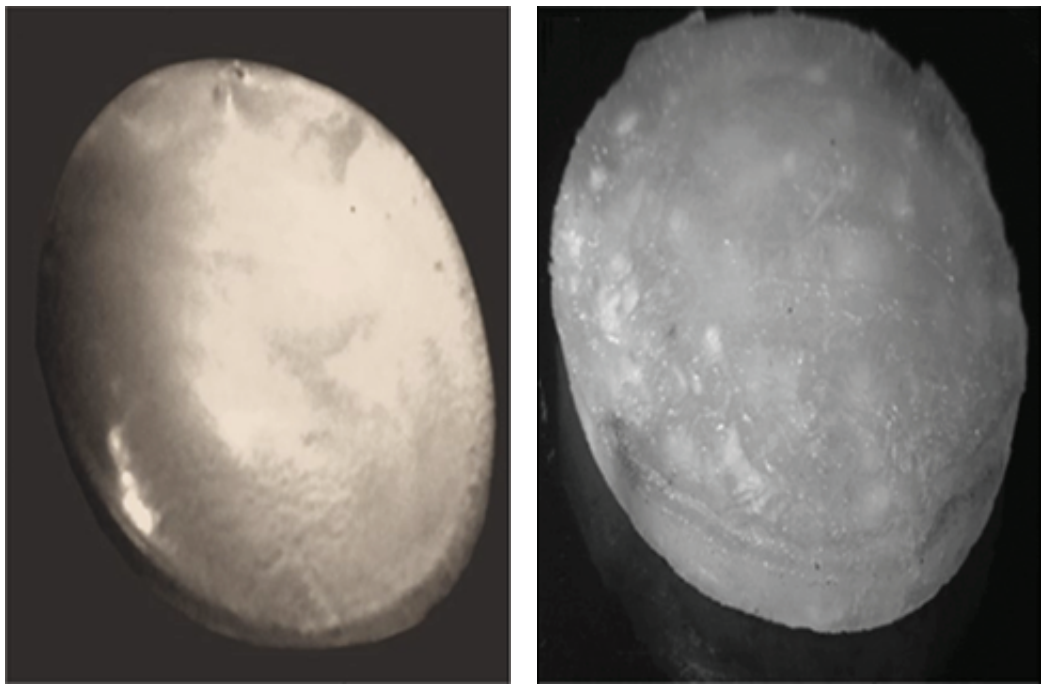
3.2. Structural Characterization of the Prepared Hydrogels. The XRD patterns of PVA, HG, HGAA, and HGE are presented in Figure 3. The absolute intensities of the main peaks corresponding to each hydrogel are presented in Table 1. The XRD pattern of PVA, used as a supplementary control, displayed a sharp peak of relatively high intensity (~582) at around 20° 2θ and peaks of much smaller intensity at around 40° 2θ (~186) and at around 44° 2θ (~160) (Figure 3). Importantly, the XRD patterns of HG (Figure 4(b)), HGAA (Figure 3(c)), and HGE (Figure 3) also elicited prominent peaks of relative intensity at around 20° 2θ (~142, ~665, and ~164, respectively), at around 40° 2θ (~36, ~175, and ~50, respectively), and at around 44° 2θ (~188, ~234, and ~124, respectively). These data confirmed the presence of PVA in their composition. Furthermore, we noticed sharp peaks at around 28° 2θ (~108, ~340, and ~92, respectively), strongly suggesting that the respective peak was due to the presence of gelatin in these three hydrogels. Nevertheless, considering that in similar previously published experimental conditions [15] the crystallinity of HG < crystallinity PVA < crystallinity of gelatin, the peak observed at around 28° 2θ due to gelatin, once cross-linked to PVA, is related to lower crystallinity. Eventually, HGAA has an XRD pattern close to the one observed for PVA, displaying a peak of low relative (and not absolute) intensity at around 28° 2θ, suggesting that acetic acid is likely to be involved in maintaining a better crystalline state of HG.

Besides, in order to ensure the cross-linking of PVA with gelatin [14] and to identify specific functional chemical groups involved in the intercalation of the plant extract components in the hydrogel, major FTIR spectral assignments obtained for HGE were compared to that of HG (Table 2).

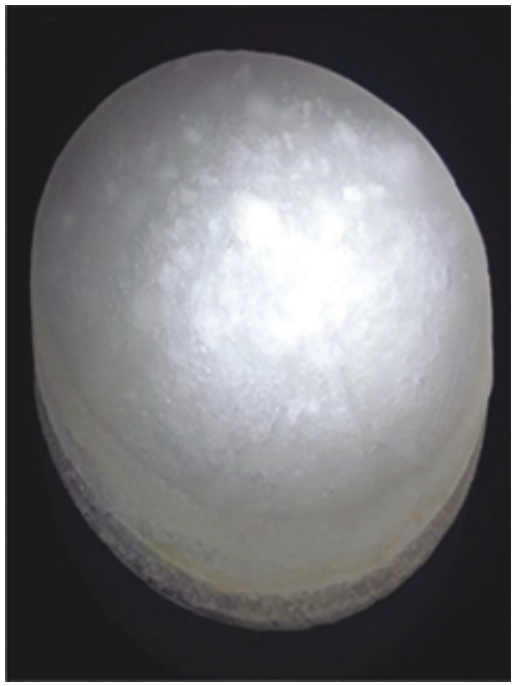
The FTIR spectrum of HG (Figure 5(a)) showed peaks of low intensity at 3615 cm^{-1} and 3222 cm^{-1} and a peak of medium intensity at 1443 cm^{-1}, which are commonly due to the stretching vibration of free O-H (hydroxyl) bonds [15] and H-H bonds and the bending vibrations of C-H bonds, respectively. Intramolecular and intermolecular hydrogen

Table 3: Water holding ability of a synthesized prepared hydrogel.

Type	0	2 hours	4 hours	6 hours	8 hours
HG	2 ± 0.07^{a}	149.67 ± 0.24^{b}	167.87 ± 0.22^{c}	168.00 ± 0.2^{c}	168.07 ± 0.11^{c}
HGE	2 ± 0.07^{a}	150.27 ± 0.18^{b}	169.97 ± 0.24^{c}	170.1 ± 0.27^{c}	170.05 ± 0.25^{c}

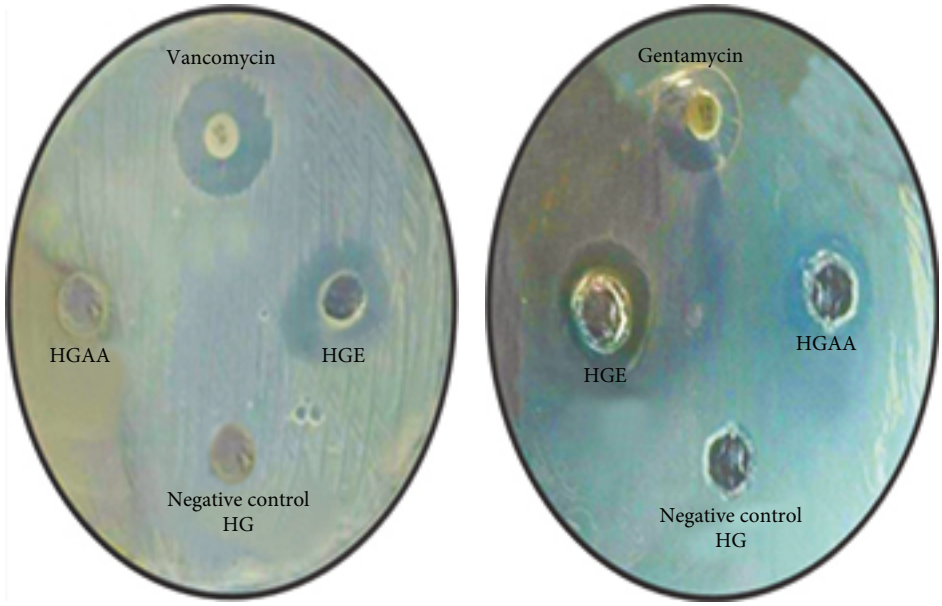


(a) (b)



(c)

Figure 6: Water holding ability of the prepared hydrogels: (a) state of HGE prior to swelling; (b) state of HGE after 2 hours of swelling; (c) state of HGE after 8 hours of swelling.



(a) (b)

Figure 7: Inhibition zones reflecting the activity of the prepared hydrogels against skin pathogens: (a) against *S. aureus*; (b) against *P. aeruginosa.*

Table 4: Inhibition zones (mm) caused by the prepared hydrogels against major skin pathogens.

	HGAA	HE	VAN	GEN	HG
S. aureus	ND*	20.07 ± 0.24^{b}	21.95 ± 0.45^{d}	—	ND*
P. aeruginosa	10.27 ± 0.38^{a}	18.00 ± 0.20^{c}	—	20.17 ± 0.31^{b}	ND*

ND* stands for "not detectable"; variables with the same letter means $p > 0.05$; variables having different letters means $p < 0.05$.

bonding are expected to occur among PVA chains due to high hydrophilic forces [26]. The peak of low intensity at 1730 cm^{-1} corresponded to a C=O ester bond, indicating the formation of an expected esterified product (i.e., esterification of all the free carboxylic groups of gelatin) [15]. Both C-H and C=C are typical of the alkane structure.

Besides, the FTIR spectrum of HGE (Figure 4) shows a peak of low intensity at 3291 cm^{-1} which indicates an asymmetrical stretching of -NH_3^+ in amino acids and a peak of strong intensity at 1652 cm^{-1} which is due to two bands stretching C=O and NH_2 in primary amides. The peak of low intensity at 1087 cm^{-1} is due to C-O stretching, and the peak of strong intensity at 943 cm^{-1} is due to CH_2 which is out-of-plane wagging in $CH{=}CH_2$ of vinyl compounds. Taken together, FTIR analysis confirmed the cross-linking between PVA and gelatin and determined a specific functional group (i.e., amine –NH_3^+) involved in the intercalation of the plant extract components in the hydrogel. FT-IR spectra analyses show that the esterification, induced by chemical cross-linking, occurred between the hydroxyl group of PVA and the carboxyl group of gelatin. Our data are concordant with other observations [16]. The infrared spectra of HGE also indicate that the plant extract did engage in –NH_3^+ bonds with the polymeric matrix, which may reduce its potential antimicrobial activity as hypothesized elsewhere [27]. Interestingly, the presence of free amine groups plays an important role in water uptake because of their hydrophilic nature [15].

3.3. Swelling Behavior of the Prepared Hydrogels. The swelling rates of HGE were compared to that of HG, and its water holding capacity (Figure 5 and Table 3) has been evaluated during 8 hours. The resulting curves obtained for HGE and HG were superposed, indicating no significant ($p > 0.05$) differences between the swelling rates (Figure 6). Thereby, in a first step, a quick increase in swelling percentage (about 150%) is observed during the first two hours of immersion/soaking which further increased gradually to about 170% for 2 hours before a saturation/equilibrium step occurred for 4 hours.

Overall, our data showed that the swelling behavior values were greater than 100% during many hours for both hydrogels, which may indicate that HGE has good biocompatibility *in vivo* and a valuable water holding capacity during the course period. Indeed, these data are of great interest in a clinical setting since swelling % decides the time of substitution of a wound dressing, which is usually short [18, 26]. Importantly, these superabsorbent hydrogels are usually biocompatible in nature and are nonirritating to soft tissues when in contact with them [28]. The potential capacity of HGE to uptake and retain wound liquids/exudates appears relatively good and sufficient for its application in a clinical setting. Indeed, in

the case of wound dressing, the shelf life of a hydrogel is not the problem, as the hydrogel is used once for a short period [29]. Eventually, we noticed that the original shape of the HG was maintained after impregnation of the plant extract in HG, which may be explained by the permanent junction-induced mechanical strength after chemical cross-linking [30].

3.4. Antimicrobial Activity of the Prepared Hydrogels. The antimicrobial activities of HGE were checked by the agar well diffusion method against two major skin pathogens, namely *S. aureus* (Figure 7(a)) and *P. aeruginosa* (Figure 7(b)). External positive controls included the glycopeptide antibiotic Vancomycin/VAN (30 μg) against *S. aureus* and the aminoglycoside antibiotic Gentamycin/GEN (10 μg) against *P. aeruginosa*. HG was used as a potential negative control, and HGAA as a potential positive control. A zone of inhibition smaller than 5 mm meant a resistance of the strain to the product (i.e., hydrogel or known antibiotic). Interestingly, HGE showed higher sensitivity zones against *S. aureus* (20 mm) and *P. aeruginosa* (18 mm) compared to that of HGAA which was only sensitive against *P. aeruginosa* (10 mm) (Table 4). The zones of inhibition for Vancomycin (22 mm) and Gentamycin (20 mm) were slightly larger compared to that of HGE when tested on *S. aureus* and *P. aeruginosa*, respectively (Table 4). Besides, both strains were resistant to HG (Figures 7(a) and 7(b) and Table 4), strongly demonstrating no inherent antimicrobial activity of the unloaded/unfilled HG. Vancomycin is a glycopeptide which is considered as the drug of choice for first-line treatment against *S. aureus*, including Methicillin Resistant *S. aureus* (MRSA) infections [31]. Gentamycin is an aminoglycoside widely recommended in the treatment of *P. aeruginosa* infections [31], since it is one of the only antibiotic agents to which strains are regularly sensitive [32, 33]. Besides, no zone of inhibition was observed using HG against the two strains. These observations indicate a sustained release of the plant extract and not an inherent antibacterial property of HG (at least when used against the selected strains).

4. Conclusions

This work was realized to explore a natural antimicrobial agent that can be used to design an original wound dressing material. In the current study, HGE was made by esterification using the conventional solution casting method. Based on its physical, behavioral, and biological characterizations, we found that HGE is able to maintain the hydrophilicity and crystallinity of HG. To the best of our knowledge, our study represents the first report about the *N. sativa* extract encapsulated into HG, which most importantly presented a good characteristic in relation to (i) the release of the active antimicrobial principle verified through a swelling test performed at dermatological pH and (ii) *in vitro* antimicrobial activity. Therefore, HGE showed a promising potential for application as a wound dressing biomaterial and may be a promising alternative formulation against at least certain Gram-positive and Gram-negative strains. To ensure its topical applicability, our ongoing project consists of further evaluating HGE regarding (i) the influence of weight ratios on properties, including gel content; (ii) its mechanical resistance/tensile strength; (iii) its thermal properties; (iv) its biodegradation rate; (v) its water vapor transmission rate; (vi) the release mechanism of extract from HG (e.g., Fickian diffusion); (vii) its bioactivity against a bunch of resistant skin strains including the determination of its bacteriostatic or microbicide effects; and (viii) its efficacy (e.g., cycles of use) and safety (e.g., cytocompatibility and biocompatibility by acute toxicity tests ex-vivo and *in vivo*).

Abbreviations

F-T:	Freezing-thawing
FTIR:	Fourier transform infrared (spectroscopy)
HG:	PVA/gelatin hydrogel
HGAA:	HG filled with acetic acid
HGE:	HG loaded with extract
MDR:	Multidrug resistant
MHB:	Mueller-Hinton broth
NA:	Not applicable
ND:	Not determined
ND*:	Not detectable
P. aeruginosa:	*Pseudomonas aeruginosa*
PEG:	Poly(ethylene glycol)
PVA:	Poly(vinyl alcohol)
S. aureus:	*Staphylococcus aureus*
SEM:	Scanning electron microscopy
TSA:	Tryptone soy agar
UV:	Ultraviolet
XRD:	X-ray powder diffraction.

Data Availability

All required data is available and can be provided on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the Higher Education Commission of Pakistan for project no. PM IPFP/HRD/-HEC/2010/1815 and the International Islamic University, Islamabad, Pakistan for supporting this research project. The authors also thank Dr. Abder Menaa, MD, for his pertinent suggestions and ideas in translational medicine.

Supplementary Materials

Figure S1: flow chart of the methodology used for the preparation of hydrogels. Figure S2: preparation of hydrogels (A). The hydrogel is peeled off with the help of forceps from a 90 mm petri dish (B). The hydrogel is saved in a ziplock packet. *(Supplementary Materials)*

References

[1] C. Mathers, D. M. Fat, and J. Boerma, *The Global Burden of Disease: 2004 Update*, World Health Organization, 2008, http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/.

[2] R. Djeribi, W. Bouchloukh, T. Jouenne, and B. Menaa, "Characterization of bacterial biofilms formed on urinary catheters," *American Journal of Infection Control*, vol. 40, no. 9, pp. 854–859, 2012.

[3] E. A. Kamoun, X. Chen, M. S. Mohy Eldin, and E.-R. S. Kenawy, "Crosslinked poly(vinyl alcohol) hydrogels for wound dressing applications: a review of remarkably blended polymers," *Arabian Journal of Chemistry*, vol. 8, no. 1, pp. 1–14, 2015.

[4] S. Bhowmick, S. Mohanty, and V. Koul, "Fabrication of transparent quaternized PVA/silver nanocomposite hydrogel and its evaluation as an antimicrobial patch for wound care systems," *Journal of Materials Science. Materials in Medicine*, vol. 27, no. 11, p. 160, 2016.

[5] A. Salomé Veiga and J. P. Schneider, "Antimicrobial hydrogels for the treatment of infection," *Biopolymers*, vol. 100, no. 6, pp. 637–644, 2013.

[6] C. Y. Loo, P. M. Young, W. H. Lee, R. Cavaliere, C. B. Whitchurch, and R. Rohanizadeh, "Non-cytotoxic silver nanoparticle-polyvinyl alcohol hydrogels with anti-biofilm activity: designed as coatings for endotracheal tube materials," *Biofouling*, vol. 30, no. 7, pp. 773–788, 2014.

[7] C. M. González-Henríquez, M. A. Sarabia-Vallejos, and J. Rodriguez-Hernandez, "Advances in the fabrication of antimicrobial hydrogels for biomedical applications," *Materials*, vol. 10, no. 3, p. 232, 2017.

[8] V. W. L. Ng, J. M. W. Chan, H. Sardon et al., "Antimicrobial hydrogels: a new weapon in the arsenal against multidrug-resistant infections," *Advanced Drug Delivery Reviews*, vol. 78, pp. 46–62, 2014.

[9] S. C. Lee, I. K. Kwon, and K. Park, "Hydrogels for delivery of bioactive agents: a historical perspective," *Advanced Drug Delivery Reviews*, vol. 65, no. 1, pp. 17–20, 2013.

[10] S. Marchesan, Y. Qu, L. J. Waddington et al., "Self-assembly of ciprofloxacin and a tripeptide into an antimicrobial nanostructured hydrogel," *Biomaterials*, vol. 34, no. 14, pp. 3678–3687, 2013.

[11] J. Nie, Z. Wang, and Q. Hu, "Difference between chitosan hydrogels via alkaline and acidic solvent systems," *Scientific Reports*, vol. 6, no. 1, article 36053, 2016.

[12] Q. Chai, Y. Jiao, and X. Yu, "Hydrogels for biomedical applications: their characteristics and the mechanisms behind them," *Gels*, vol. 3, no. 1, p. 6, 2017.

[13] A. Ahmad, A. Husain, M. Mujeeb et al., "A review on therapeutic potential of *Nigella sativa*: a miracle herb," *Asian Pacific Journal of Tropical Biomedicine*, vol. 3, no. 5, pp. 337–352, 2013.

[14] B. Uzair, A. Hameed, S. Nazir et al., "Synergism between *Nigella sativa* seeds extract and synthetic antibiotics against Mec a gene positive human strains of *Staphylococcus aureus*," *International Journal of Pharmacology*, vol. 13, no. 8, pp. 958–968, 2017.

[15] K. Pal, A. K. Banthia, and D. K. Majumdar, "Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications," *AAPS PharmSciTech*, vol. 8, no. 1, pp. E142–E146, 2007.

[16] G.-Y. Zuo, X.-J. Zhang, C.-X. Yang, J. Han, G.-C. Wang, and Z.-Q. Bian, "Evaluation of traditional Chinese medicinal plants for anti-MRSA activity with reference to the treatment record of infectious diseases," *Molecules*, vol. 17, no. 3, pp. 2955–2967, 2012.

[17] J. E. Welton, *SEM Petrology Atlas, Chevron Oil Field Research Company, Methods in Exploration Series No. 4*, The American Association of Petroleum Geologists, Oklahoma, OK, USA, 2003.

[18] M. Kokabi, M. Sirousazar, and Z. M. Hassan, "PVA-clay nanocomposite hydrogels for wound dressing," *European Polymer Journal*, vol. 43, no. 3, pp. 773–781, 2007.

[19] M. A. Aziz, J. D. Cabral, H. J. L. Brooks, S. C. Moratti, and L. R. Hanton, "Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use," *Antimicrobial Agents and Chemotherapy*, vol. 56, no. 1, pp. 280–287, 2012.

[20] C. Valgas, S. M. d. Souza, E. F. A. Smânia, and A. Smânia Jr., "Screening methods to determine antibacterial activity of natural products," *Brazilian Journal of Microbiology*, vol. 38, no. 2, pp. 369–380, 2007.

[21] A. M. Kearns, M. Ganner, and A. Holmes, "The "Oxford *Staphylococcus*": a note of caution," *Journal of Antimicrobial Chemotherapy*, vol. 58, no. 2, pp. 480-481, 2006.

[22] CLSI, "Performance standards for antimicrobial disk susceptibility tests, approved standard," in *CLSI Document M02-A11*, pp. 1–58, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 7th edition, 2012.

[23] E. M. Ahmed, "Hydrogel: preparation, characterization, and applications: a review," *Journal of Advanced Research*, vol. 6, no. 2, pp. 105–121, 2015.

[24] S. Moscato, F. Ronca, D. Campani, and S. Danti, "Poly(vinyl alcohol)/gelatin hydrogels cultured with HepG2 cells as a 3D model of hepatocellular carcinoma: a morphological study," *Journal of Functional Biomaterials*, vol. 6, no. 1, pp. 16–32, 2015.

[25] E. A. Kamoun, E.-R. S. Kenawy, and X. Chen, "A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings," *Journal of Advanced Research*, vol. 8, no. 3, pp. 217–233, 2017.

[26] E. F. dos Reis, F. S. Campos, A. P. Lage et al., "Synthesis and characterization of poly(vinyl alcohol) hydrogels and hybrids for rMPB70 protein adsorption," *Materials Research*, vol. 9, no. 2, pp. 185–191, 2006.

[27] K. S. P. Jodar, V. M. Balcao, M. V. Chaud et al., "Development and characterization of a hydrogel containing silver sulfadiazine for antimicrobial topical applications," *Journal of Pharmaceutical Sciences*, vol. 104, no. 7, pp. 2241–2254, 2015.

[28] P. Gupta, K. Vermani, and S. Garg, "Hydrogels: from controlled release to pH-responsive drug delivery," *Drug Discovery Today*, vol. 7, no. 10, pp. 569–579, 2002.

[29] A. Hassan, M. B. K. Niazi, A. Hussain, S. Farrukh, and T. Ahmad, "Development of anti-bacterial PVA/starch based hydrogel membrane for wound dressing," *Journal of Polymers and the Environment*, vol. 26, no. 1, pp. 235–243, 2018.

[30] M. C. Hacker and A. G. Mikos, *Synthetic Polymers, Principles of Regenerative Medicine*, Elsiever, 2nd edition, 2011.

[31] C. N. Chaudhari, K. Tandel, N. Grover et al., "In vitro vancomycin susceptibility amongst methicillin resistant *Staphylococcus aureus*," *Medical Journal Armed Forces India*, vol. 70, no. 3, pp. 215–219, 2014.

[32] Y. Morita, J. Tomida, and Y. Kawamura, "Responses of *Pseudomonas aeruginosa* to antimicrobials," *Frontiers in Microbiology*, vol. 4, 2014.

[33] T. S. Sivanmaliappan and M. Sevanan, "Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers," *International Journal of Microbiology*, vol. 2011, Article ID 605195, 4 pages, 2011.