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Gelatin-PVA-AgNPs Triad Composite as Wound Healing Hydrogel with Wounded Skin Surface Protective Efficiency

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ABSTRACT

The development of non-toxic, biocompatible and antibacterial hydrogels are ideal biomaterials as wound dressing material for promoting wound healing. Thus, we report the development of a low-cost biomacromolecule-based triad nanocomposite hydrogel. Gelatin, a collagen hydrolysate based biomacromolecule, polyvinyl alcohol (PVA), and green-synthesized silver nanoparticles (AgNPs) form the hydrogels for wound dressing application. Gelatin enhances the efficiency of the hydrogel blended with synthetic macromolecule PVA. Silver nanoparticles (AgNPs) synthesized using *Musa balbisiana* (Bhimkol) peels aqueous extract were incorporated into the PVA-Gelatin hydrogel blend. The developed PVA-gelatin-AgNPs triad nanocomposite hydrogel films were characterized and studied by various spectroscopic and analytical techniques. The incorporation of AgNPs into the gelatin-PVA blend offers better stability, improved water vapour transmission rate (WVTR), surface contact angle, swelling ratio and mechanical properties compared to the native PVA-gelatin blend. The PVA-gelatin-AgNPs triad bionanocomposite hydrogel films showed potent antibacterial efficiency against the commonly present microbes in wounds i.e., *E.coli* and *S. aureus*. The hydrogel films showed no toxicity against the BJ normal human foreskin fibroblasts cells. Our composite hydrogel was found to accelerate wound healing, efficiently protect the wounded skin surface against exudate accumulation/dehydration, and prevent bacterial growth and infection. Thus, these gelatin-PVA-AgNPs triad nanocomposite hydrogels acts as ideal for wound dressing applications.

Keywords: Gelatin biomacromolecule, Gelatin-PVA-AgNP Nanocomposite Hydrogel Dressing, Green synthesized AgNPs, Antibacterial Efficiency, Wound Healing, Surface protective efficiency.

1. INTRODUCTION

Wound infection prevention and moisture retention at the wound site are the two main aspects of wound healing[1]. An ideal wound dressing should be biocompatible, non-toxic, absorbs wound exudate and have timely gas exchange. Wound dressings stimulate wound healing by decreasing the pain and reducing scar formation. Antibacterial hydrogels are considered ideal wound dressing materials for preventing wound infection and providing a super moist environment at the wound site[2]. Hydrogels absorb excess exudates of the wound, offer a moist environment, prevent cellular dehydration and promote quicker healing by building a protective barrier at the wound interface[3]. Hydrogels are very soft materials that are advantageous over the available wound dressing materials. Both natural and synthetic polymers or composites are used to synthesize hydrogels. These are often helpful in biomedicine research, including controlled drug and protein delivery, regenerative medicine, and tissue engineering[4].

Biological macromolecules have attracted significant research interest in designing and blending hydrogels for clinical applications, such as wound healing. Collagen is a natural biomacromolecule that, when hydrolyzed, afforded gelatin biomolecule with the strong gelating property. Thus, the presence of gelatin enhances the strength and efficiency of hydrogels for their respective application[5]. Gelatin is a natural macromolecule, a denatured form of collagen protein, used primarily in biomedical applications. It is widely used in biomedicine, primarily in preparing adhesives, plasma-expander wound dressing material, or hydrogel pads as absorbent. It exhibits high hemostatic properties. It causes activation of macrophages and is thus used in many wound dressing applications. Gelatin forms powerful gels in the presence of a high level of

pyrrolidines via the formation of triple helix structure. It is biocompatible, nontoxic, biodegradable. It has strong associability with other biomaterials via H-bonding interaction. Thus, all these properties make gelatin a suitable material for preparing hydrogel when blended with other suitable polymers, such as PVA[6,7]. Gelatin protein combined with other polymeric materials based-hydrogel possessing antimicrobial property has gained popularity in biomaterials research. These materials with improved sterility and longevity find wide applications in medical science, especially to overcome the problem of frequent change of dressings in the wound sites[8,9].

Poly (vinyl alcohol) acts as an excellent base for wound dressing material among the various hydrogel materials used. PVA is biocompatible, non-toxic, non-carcinogenic and possesses good elasticity, good swelling properties and high physical cross-linking efficiency. Being hydrophilic in nature, PVA has very good water retention capacity[10]. The hydrogen bonding among the hydroxyl groups of PVA polymer influences the water solubility, crystallinity range, and high crystal modulus. Semi-crystalline PVA has both crystalline and amorphous regions, which causes the interfacial effect. However, PVA is usually low in electrical conductivity, which can be enhanced by blending it with other polymers[6]. To improve biocompatibility and increase wound healing capacity other natural polymers such as chitosan and its derivatives, starch, gelatin and alginate are generally added with PVA[8].

Thus, the PVA-Gelatin blends are widely used to prepare the hydrogel. They possess three-dimensional crosslinked networks of polymers that are generally hydrophilic and biocompatible. Polymeric hydrogels have good swelling properties while remaining undissolved in water at physiological pH and temperature[10]. However, incorporating an antibacterial agent is an excellent option to improve the wound healing property and the quality and sterility of the

hydrogels. When combined with AgNPs, the antibacterial properties of the polymeric materials enhances. *In situ* biomimetic synthesis of AgNPs within PVA-sericin hydrogel was established by synthesizing the AgNPs by the redox property of tyrosine residues in sericin without adding any other chemicals. Sericin is a natural protein from silkworm cocoon used to develop biodegradable and biocompatible skin-repairing materials. These polymeric hydrogels developed, exhibited high porosity, good mechanical properties, and wettability of the hydrogels. The composites had good biocompatibility and showed antibacterial activity against *S. aureus*, *E.coli* and *P. aeruginosa*[11]. Due to the exploitation of antibiotics, inorganic nanoparticles, especially AgNPs, have received attention for their broad-spectrum antimicrobial activity. AgNPs were also synthesized *in situ* in sericin by its phenolic hydroxyl group of tyrosine to develop novel hydrogel wound dressing material using sodium alginate(SA/Se) network. Calcium ions were the crosslinking agents between sodium alginate and sericin. These hybrid hydrogels exhibited effective antibacterial activity, resulting in bacterial membrane disruption and bacterial lysis and death[2]. The antimicrobial activity of AgNPs is associated with adhesion onto the bacteria or microbes' cell walls and cell surfaces. It also helps damage the cytoplasmic membrane and other intracellular structures, inhibits DNA replication, and interrupt ATP production leading to respiratory disruption[12]. AgNPs induce the generation of reactive oxygen species (ROS) and free radicals, causing oxidative stress and cellular toxicity to the bacteria. Moreover, AgNPs are also known to modulate the signal transduction pathways[13].

In this respect, the silver nanoparticles (AgNPs) are the choice of interest for improving the antibacterial activity of the hydrogel[14,15]. Polydopamine (PDA) was coated on the surface of sericin and agar composite films, which synthesized high-density AgNPs to form antibacterial AgNPs-PDA-SS/Agar films. These composite films showed good hydrophilicity, mechanical

properties and antibacterial ability. They also had admirable cytocompatibility on the fibroblast NIH/3T3 cells, which showed as a potential for wound healing[16]. Again, in another study, ZnO and AgNPs were self-assembled layer by layer on sericin-agarose composite films using polydopamine to attain better antimicrobial activity. The composite films showed desirable mechanical and hydrophilicity and exhibited better antimicrobial activity[17].

Therefore, a material formulation based on PVE-gelatin-blend mixed with AgNPs in lower concentrations would provide promising, easy-to-use hydrogel with potent antibacterial activity and wound dressing property. Thus, we utilized our previously reported green-synthesized AgNPs[15] to prepare triad composite polymeric hydrogel with wound healing/dressing property. AgNPs were green synthesized using banana peels of *Musa balbisiana* variety in a very eco-friendly and cost-effective synthesis methodology. *Musa balbisiana* peels have high phenolic compounds, which results in their antimicrobial and antioxidant properties, increasing their medicinal value. Thus, the AgNPs synthesized using the aqueous extract of *Musa balbisiana* peels have great potential in wound healing when combined with polymer blend or composites, mainly by reducing the complexity of the hydrogel preparation and providing antibacterial properties. We envisioned that PVA-gelatin polymer blend embedded with AgNPs would bring the suitable mechanical property of the hydrogel, which is considered one of the most important properties that a wound dressing material should have to protect wounds[8,12]. Furthermore, the presence of AgNPs would be effective in faster wound healing by reducing infections caused by bacteria reducing scars and inflammation. Although few reports of green synthesis of silver nanoparticles exist, the applications are not documented[18,19].

Thus, as part of our continuous effort to synthesize bionanomaterial for biomedical applications, we, in this study, report the green synthesis of AgNPs and PVA-Gelatin-AgNPs triad composite

polymer hydrogel as wound dressing material. Out of several prepared hydrogel films, the gel composed of 3.5% PVA-0.5% gelatin-2.0% AgNPs, proved to exhibit good mechanical strength compared to the native PVA-gelatin blend with potent antibacterial activity, nontoxicity, and efficient wound healing/dressing property.

2. RESULTS AND DISCUSSION

2.1. Preparation of Polymer Composite Hydrogels and Silver Nanocomposite Polymer Hydrogels:

The preparation of PVA and PVA-gelatin hydrogel films involves the utilization of varying concentrations and proportions (% w/w ratio) of polyvinyl alcohol (PVA) and gelatin. The solution casting method was used for the preparation of PVA-gelatin films[6]. Thus, 4 grams of PVA was added slowly in 100 mL distilled water and stirred continuously for 2 hours in a magnetic stirrer at 60°C, making it 4% PVA solution (control). After PVA dissolved entirely in water, it was sonicated for 5 minutes to remove the air bubbles. The solution was then cast on clean and sterile Petri plates and kept in a hot air oven at 60°C for slow evaporation for a few hours. Similarly, 4 grams of gelatin was added slowly in 100 mL distilled water and stirred continuously at 60°C, making it a 4% Gelatin(control) solution. The other combinations of PVA and gelatin films were prepared to keep the PVA amount constant at 3.5%, and the amount of gelatin was varied from 0.5% to 3.5% in w/w ratio. Thus, 3.5 grams of PVA was added to 50 mL milli-Q water and allowed to stir at 60°C to dissolve completely. Gelatin was added to another 50 mL of water in varying quantities. The gelatin solution was added to the polymeric solution with continuous stirring for an esterification reaction between PVA and gelatin. The solution obtained was sonicated for 5 minutes and then cast into sterile Petri plates. The various combinations of the films thus were made (a) 4% PVA (Control), (b) 4% Gelatin (Control), (c) 3.5% PVA + 0.5% gelatin, (d) 3.5% PVA + 1.0% gelatin, (e) 3.5% PVA + 1.5% gelatin, (f) 3.5% PVA + 2.0% gelatin, (g) 3.5% PVA

+ 2.5% gelatin, (h) 3.5% PVA + 3.0% gelatin, and (i) 3.5% PVA + 3.5% gelatin. The casting method prepared thin films of PVA, Gelatin, and PVA-Gelatin hydrogels. After drying the hydrogel films in a hot air oven at 60°C for a few hours, these hydrogel films were peeled off and stored in a vacuum desiccator to further analysis.

Next, we prepared silver nanoparticles incorporated PVA-Gelatin hydrogel films. Thus, 1 mM concentration of green synthesized silver nanoparticle (See the Experimental Section) was mixed into the PVA-Gelatin polymer hydrogel following a solution casting method. Maintaining the PVA-gelatin ratio [3.5: 0.5 to 0.5: 3.5 % (w/w ratio)], various %v/w (0.5%, 1.0%, 1.5% and 2.0%) concentration of 1 mM green synthesized AgNPs were mixed in each hydrogel. The concentration of silver nanoparticles is kept constant in each solution, and the quantity of silver nanoparticles in mL was taken in the casting solution. After completely evaporating water in a hot air oven, thin films of PVA-gelatin-AgNP hydrogel remained on the Petri dishes peeled off and stored in a vacuum desiccator for further characterizations.

2.2.Study of Swelling Properties of The NC Hydrogel Films

Excellent swelling behavior of a hydrogel is considered one of the most important properties for the hydrogel to absorb a large quantity of water in the blood to help cease bleeding. Swelling property maintains the state of the hydrogel when it absorbs water and the capacity to hold water onto it. Further, to serve as a good dressing material, the hydrogels should reach a swelling and de-swelling equilibrium in a short period with good permeability to nutrients[20]. Therefore, for our triad nanocomposite hydrogel films, the swelling behaviour was tested for 500 minutes with 30 minutes intervals for each hydrogel films.

Thus, we studied the swelling kinetics of 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0% gelatin hydrogel films incorporated with green synthesized AgNPs in various ratios (**Figure 1(i)**-

(ii)). The PVA hydrogel film and PVA-gelatin blend hydrogel films showed good swelling behaviour since both the gelatin and PVA are hydrophilic in nature. Thus, from the **figure 1(i)** it is clear that all the triad PVA-gelatin-AgNP hydrogel films achieved swelling equilibrium within 30 minutes. The water uptake capacity was found to be 654% for (a) 3.5% PVA + 0.5% gelatin(Control), 657% for (b) 3.5% PVA + 0.5% gelatin + 0.5% AgNP, 675% for (c) 3.5% PVA + 0.5% gelatin + 1.0% AgNP, 684% for (d) 3.5% PVA + 0.5% gelatin + 1.5% AgNP and 753% for (e) 3.5% PVA + 0.5% gelatin + 2.0% AgNP respectively, at equilibrium. Hence, we found that all the AgNPs incorporated hydrogel films had more water uptake capacity than that of the control polymer composite (PVA-gelatin) hydrogel films. Furthermore, the triad PVA-gelatin-AgNP hydrogel film with compositions, 3.5% PVA + 0.5% gelatin + 2.0% AgNP, exhibited highest water uptake capacity among all at swelling equilibrium.

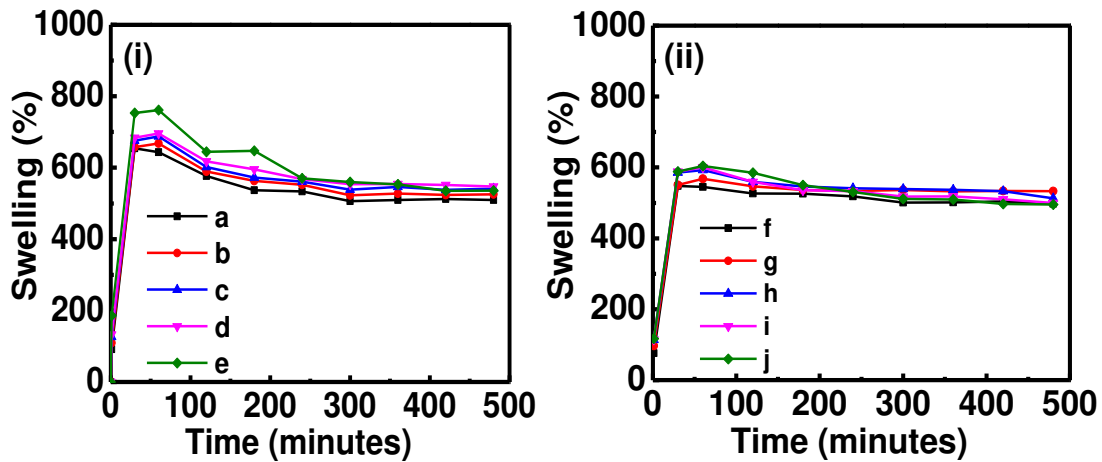


Figure 1. (i) Swelling kinetics of (a) 3.5% PVA + 0.5% gelatin (Control), (b) 3.5% PVA + 0.5% gelatin + 0.5% AgNP, (c) 3.5% PVA + 0.5% gelatin + 1.0% AgNP, (d) 3.5% PVA + 0.5% gelatin + 1.5% AgNP and (e) 3.5% PVA + 0.5% gelatin + 2.0% AgNP hydrogel films and (ii) Swelling kinetics of (f) 3.5% PVA + 1.0% gelatin (Control), (g) 3.5% PVA + 1.0% gelatin + 0.5% AgNP, (h) 3.5% PVA + 1.0% gelatin + 1.0% AgNP, (i) 3.5% PVA + 1.0% gelatin + 1.5% AgNP and (j) 588.30% of 3.5% PVA + 1.0% gelatin + 2.0% AgNPs hydrogel films respectively.

Again, from the **figure 1(ii)** it is clear that all the PVA-gelatin-AgNP hydrogel films achieved swelling equilibrium within 30 minutes and the water uptake capacity was found to be 548% for (f) 3.5% PVA + 1.0% gelatin (Control), 550% for (g) 3.5% PVA + 1.0% gelatin + 0.5% AgNP, 586% for (h) 3.5% PVA + 1.0% gelatin + 1.0% AgNP, 588% for (i) 3.5% PVA + 1.0% gelatin + 1.5% AgNP and 588% for (j) 3.5% PVA + 1.0% gelatin + 2.0% AgNP respectively at equilibrium. Thus, we observed that the triad nanocomposite hydrogel film with composition, (j) 3.5% PVA + 1.0% gelatin + 2.0% AgNP, or (i) 3.5% PVA + 1.0% gelatin + 1.5% AgNP exhibited the highest swelling equilibrium with water uptake capacity of 588%. However, this is less than that was shown by the film with composition (e) 3.5% PVA + 0.5% gelatin + 2.0% AgNP. In this case also, we found that all the polymer hydrogel nanocomposite films had more water uptake capacity than that of the control polymer hydrogel film.

Among all the PVA/Gelatin hydrogel films, 3.5% PVA + 0.5% gelatin blend and 3.5% PVA + 1.0% gelatin blend hydrogels exhibited better water uptake capacity. These two blend films were considered for preparing triad nanocomposite hydrogel films embedding varying concentrations of AgNPs and further characterization and studies. **Table 1** represents the swelling ratio or the water uptake capacity after 30 minutes of swelling of the hydrogel combinations of 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0% gelatin hydrogel films after incorporating AgNPs in various concentrations.

Table 1. The swelling ratio of the nanocomposite (NC) hydrogel films after 30 minutes.

Sample no.	PVA/Gelatin/AgNP hydrogel films	Water uptake capacity or Swelling ratio(%)
(a)	3.5% PVA + 0.5% gelatin(Control)	654%
(b)	3.5% PVA + 0.5% gelatin + 0.5% AgNP	657%
(c)	3.5% PVA + 0.5% gelatin + 1.0% AgNP	675%
(d)	3.5% PVA + 0.5% gelatin + 1.5% AgNP	684%
(e)	3.5% PVA + 0.5% gelatin + 2.0% AgNP	753%
(f)	3.5% PVA + 1.0% gelatin(Control)	548%
(g)	3.5% PVA + 1.0% gelatin + 0.5% AgNP	550%
(h)	3.5% PVA + 1.0% gelatin + 1.0% AgNP	586%
(i)	3.5% PVA + 1.0% gelatin + 1.5% AgNP	588%
(j)	3.5% PVA + 1.0% gelatin + 2.0% AgNP	588%

Thus, from the swelling kinetic study, it is clear that all the triad polymer hydrogel nanocomposite films with low gelatin and high PVA ratio exhibited faster absorption rates, which would help to provide a wet environment for faster recovery of wounds[21]. Especially the triad polymeric hydrogel nanocomposite films with composition 3.5% PVA + 0.5% gelatin + 2.0% AgNP expectedly would absorb a large amount of wound exudate with a capacity of holding water in the hydrogel for a longer time.

2.3.Study of Mechanical Properties of The NC Hydrogel Films

The mechanical properties of a dressing material are considered one of the most important factors for their use in protecting wounds. Thus, we next measured the mechanical strength of the hydrogel films and analyzed the tensile strength and elongation-at-break values of the prepared wound dressings triad composite hydrogel films. **Figure 2** presents the stress vs strain curves of 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0 % gelatin hydrogel films incorporated with green

synthesized AgNPs in various ratios respectively. The mechanical strength of 3.5% PVA + 0.5% gelatin + 2.0% AgNP hydrogel film showed highest among all the other hydrogel films with a tensile strength of 112.32 MPa and elongation-at-break as 7.5% (**figure 2(i)**). The mechanical strength of the 3.5% PVA + 1.0% gelatin + 1.0% AgNP hydrogel film was the second-highest among the other hydrogel films with a tensile strength of 99.44 MPa and elongation-at-break as 4.98% (**figure 2(ii)**). In both cases, the polymer hydrogel films incorporated with green synthesized AgNPs showed enhanced mechanical properties than the control polymeric hydrogel films. **Table 2** summarizes the tensile strength and elongation-at-break values for all the combinations of polymeric hydrogel films incorporated with and without AgNPs.

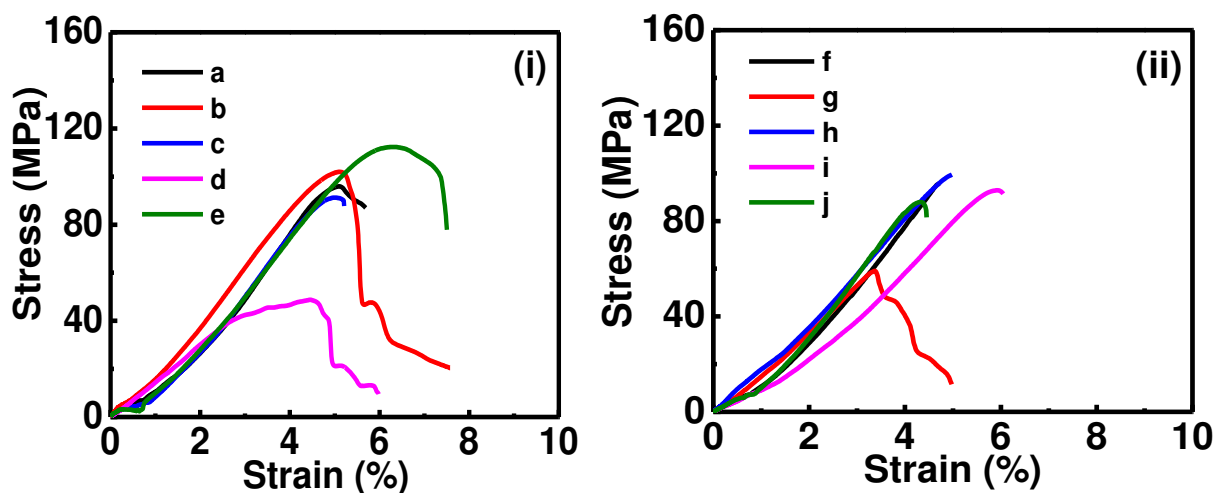


Figure 2. Tensile strength of (i) 3.5% PVA+ 0.5% gelatin (Control) and its other combinations with 0.5-2.0 % AgNPs (a-e) hydrogel films respectively; (ii) 3.5% PVA + 1.0% gelatin (Control) and its combinations with 0.5-2.0% AgNP (f-j) hydrogel films respectively.

Table 2. Tensile strength and elongation-at-break of the PVA-gelatin-AgNP hydrogel films.

Sample no.	PVA/Gelatin/AgNP hydrogel films	Tensile strength (MPa)	Tensile Elongation at Break (%)
(a)	3.5% PVA + 0.5% gelatin(Control)	95.92	5.69
(b)	3.5% PVA + 0.5% gelatin + 0.5% AgNP	101.95	7.57
(c)	3.5% PVA + 0.5% gelatin + 1.0% AgNP	91.22	5.21
(d)	3.5% PVA + 0.5% gelatin + 1.5% AgNP	48.77	5.98
(e)	3.5% PVA + 0.5% gelatin + 2.0% AgNP	112.32	7.5
(f)	3.5% PVA + 1.0% gelatin(Control)	95.77	4.72
(g)	3.5% PVA + 1.0% gelatin + 0.5% AgNP	59.05	4.97
(h)	3.5% PVA + 1.0% gelatin + 1.0% AgNP	99.44	4.98
(i)	3.5% PVA + 1.0% gelatin + 1.5% AgNP	92.88	6.05
(j)	3.5% PVA + 1.0% gelatin + 2.0% AgNP	87.93	4.45

2.4.Fourier Transform Infrared (FT-IR) Spectroscopic Character of the NC-Hydrogel Films

FT-IR spectral study provided precise information on the molecular structure of a material. The position of the peaks, intensity and width of the bands might be subjected to change due to environmental change and conformation of macromolecules at the molecular level. When different polymers are compatible, intermolecular interactions may occur. Thus, it is advantageous for studying polymer compatibility using FTIR spectroscopy. Thus, the recorded FTIR spectrum for the blends of polymers appears different from that of pure polymers[6]. **Figure 3(i)** shows the FTIR spectrum of (a) 3.5% PVA+ 0.5% gelatin (control), (b) 3.5% PVA + 0.5% gelatin + 0.5% AgNP, (c) 3.5% PVA + 0.5% gelatin + 1.0% AgNP, (d) 3.5% PVA + 0.5% gelatin + 1.5% AgNP and (e) 3.5% PVA + 0.5% gelatin + 2.0% AgNP hydrogel films respectively. **Figure 3(ii)**

represents the FTIR spectrum of (f) 3.5% PVA + 1.0% gelatin(control), (g) 3.5% PVA + 1.0% gelatin + 0.5% AgNP, (h) 3.5% PVA + 1.0% gelatin + 1.0% AgNP, (i) 3.5% PVA + 1.0% gelatin + 1.5% AgNP and (j) 3.5% PVA + 1.0% gelatin + 2.0% AgNP hydrogel films. From the FTIR spectra of the PVA-Gelatin hydrogel films, bands at 916 and 850 cm^{-1} confirm the presence of PVA skeletal vibration. The band at 1096 cm^{-1} represents C-O stretching and O-H bending. Also, C-C and C-O stretching vibrations occur at 1144 cm^{-1} and the small bands at 1438 and 1378 cm^{-1} correspond to the bending and wagging of CH_2 and C-H vibrations. A broad peak occurs at around 3279 cm^{-1} , which indicates hydroxyl group stretching vibration. Also, the smaller peaks at around 2923 cm^{-1} and 2850 cm^{-1} indicate C-H asymmetric stretching vibrations. The band at around 1640 cm^{-1} corresponds to the acetyl C=O stretching vibrations. Again, due to the presence of gelatin, the region between 3000-3600 cm^{-1} and between 1100-1700 cm^{-1} gives potential peaks, which indicates N-H stretching vibrations that are hydrogen-bonded. The peak at 3286 cm^{-1} corresponds to the presence of a hydroxyl group with the polymeric association and signifies a secondary amide. Again, the C-O stretching of secondary alcoholic groups is indicated by the peak at 1088 cm^{-1} and the peak at 1635 cm^{-1} corresponds to the presence of the secondary amide group. The peak at 2932 cm^{-1} corresponds to the occurrence of a hydrocarbon chromophore in the esterified product. So this esterified product has a secondary alcoholic group and secondary amide groups along with the hydrocarbon chain. Thus, the FTIR study supported a good interaction between the two polymers, *i.e.*, gelatin and PVA, in the polymer blends[6].

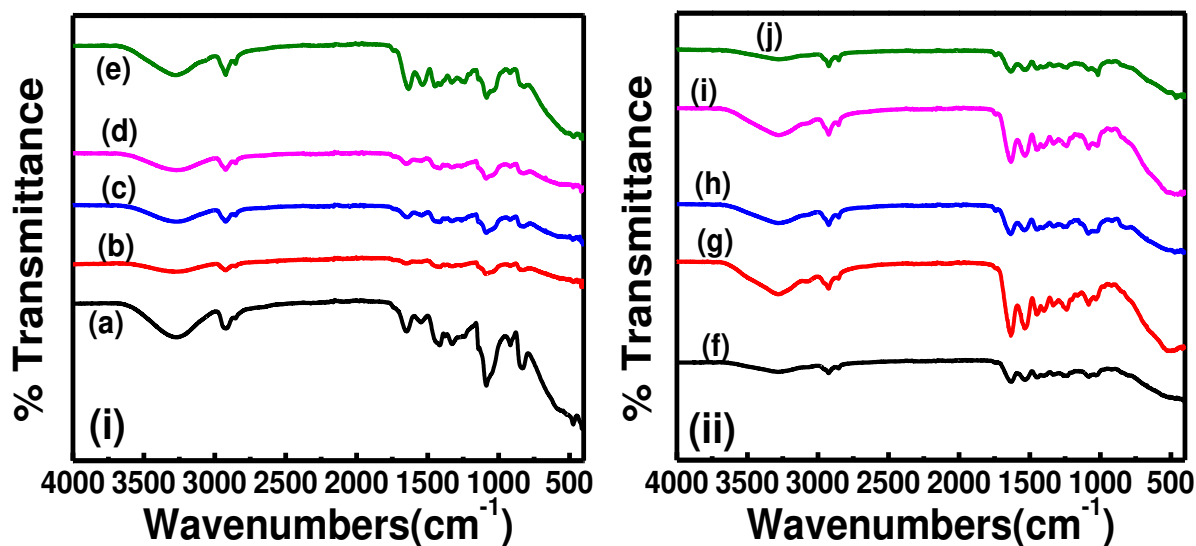


Figure 3. FT-IR spectrum of (i) 3.5% PVA+ 0.5% gelatin (Control) and its other combinations with 0.5-2.0 % AgNPs(a-e) hydrogel films respectively; (ii) 3.5% PVA + 1.0% gelatin(Control) and its combinations with 0.5-2.0% AgNP(f-j) hydrogel films respectively.

The FTIR spectrums of both the combinations of triad polymeric nanocomposite (NC) hydrogel films (PVA-gelatin-AgNPs hydrogel films or NC-hydrogel films), showed the strong and broad absorption peak at around 3284-3443 cm^{-1} corresponds to the N-H stretching vibrations of amines. This peak corresponds to the O-H (H-bonded) stretching vibrations of phenols and carboxylic acids. Again, the peak observed at 1633 cm^{-1} attributes to the C=O stretching in carboxyl or C=N bending vibration in the amide group. The peak at 667-598 cm^{-1} corresponds to the C-H stretching of the aromatic group. The shifts in the peak highlight the involvement of functional groups of bhimkol (*Musa balbisiana*) peel extract in the reduction of silver salt (Ag^+) to metallic silver (Ag^0). Thus, the study observed that the NC-hydrogel films' chemical structures were well preserved without forming any new chemical bonds during the hydrogel-forming process, indicating that these hydrogel films are formed by crosslinking[22]. Further, the FTIR study revealed that the nanoparticles remained embedded within the polymeric network with different FTIR signatures from the polymer-hydrogel films without AgNPs.

2.5. Study of Water Vapour Transmission Rate (WVTR)

In wound healing, the water vapour transmission rate (WVTR) of the uppermost surface of a membrane determines the wound's moist environment, which is one of the most important criteria for suitable wound dressing materials. Lower WVTR of a membrane or hydrogel film material generally causes retention of the exudate builds up, leading to softening of the healthy tissues of the surroundings of the wound area. This ultimately triggers pain in the patient's wounded area and helps increase the bacterial growth in the wound area. On the contrary, higher WVTR of a membrane or material leads to excessive water loss and dehydrates the wound, which ultimately initiates the formation of scar or sticking of the dressing materials to the wounded skin[23]. The reported standard water vapour transmission for skin without any injury, skin with first-degree burn and skin with granulated injury are about 200, 300 and 5000 g/m².day, respectively. Thus, an ideal wound dressing material should prevent water loss and dryness. Further, such materials should also avoid the formation of exudates in the injury site. An perfect wound healing dressing material must have a WVTR of about 2000-2500 g/m².day[24].

Table 3. WVTR of the hydrogel films after 24 hours.

Sample no.	PVA/Gelatin/AgNP hydrogel films	Water Vapour Transmission Rate (g.m ⁻² .24 hr) (WVTR±S.D)
(a)	3.5% PVA + 0.5% gelatin(control)	1248.75±90.015
(b)	3.5% PVA + 0.5% gelatin + 0.5% AgNP	1426.45±75.873
(c)	3.5% PVA + 0.5% gelatin + 1.0% AgNP	1824.1±139.159
(d)	3.5% PVA + 0.5% gelatin + 1.5% AgNP	2095.9±126.855
(e)	3.5% PVA + 0.5% gelatin + 2.0% AgNP	2239±98.571
(f)	3.5% PVA + 1.0% gelatin(control)	1072.9±48.507
(g)	3.5% PVA + 1.0% gelatin + 0.5% AgNP	1567.8±77.216
(h)	3.5% PVA + 1.0% gelatin + 1.0% AgNP	1909.8±79.196
(i)	3.5% PVA + 1.0% gelatin + 1.5% AgNP	2010.95±32.598
(j)	3.5% PVA + 1.0% gelatin + 2.0% AgNP	2232.15±74.034

PVA: Poly (vinyl alcohol), AgNP: Green synthesized Silver Nanoparticles, WVTR: Water vapour transmission rate, S.D: Standard deviation, where *p-value* <0.05 respectively.

Therefore, we studied the water vapour transmission rate (WVTR) of all the NC-hydrogel films as prepared. **Table 3** represents the WVTR of the hydrogel samples after 24 hours duration. Thus, the WVTR of the standard film, 3.5% PVA + 0.5% gelatin + 2.0% AgNP was found as 2239 g.m⁻².d⁻¹ and that for 3.5% PVA + 1.0% gelatin + 2.0% AgNP was 2232.15 g.m⁻².d⁻¹. These results showed an improvement in WVTR when compared with their corresponding control polymer blends, 3.5% PVA + 0.5% gelatin (Control; WVTR = 1248.75 g.m⁻².d⁻¹) and 3.5% PVA + 1.0% gelatin (Control; WVTR = 1072.9 g.m⁻².d⁻¹) (**table 3**).

Figure 4(i)-(ii) showed a graphical representation of the comparative WVTR values of all the hydrogel films. In **figure 4(i)** 3.5% PVA + 0.5% gelatin(control) hydrogel film and other formulations of 3.5% PVA + 0.5% gelatin hydrogel films after addition of silver nanoparticles are compared whereas in **figure 4(ii)** 3.5% PVA + 1.0% gelatin (control) hydrogel film and other formulations of 3.5% PVA + 1.0% gelatin hydrogel films after addition of AgNPs are compared. From both the figures, the WVTR of all the polymer nanocomposite hydrogel films increased with an increase in the AgNP content. Based on the results, we observed that the NC-hydrogel films containing 2.0% AgNP and exhibiting WVTR of 2239 g.m⁻².d⁻¹ are ideal wound dressing materials.

Thus, these films would be ideal for maintaining optimum moisture content during the wound healing[25].

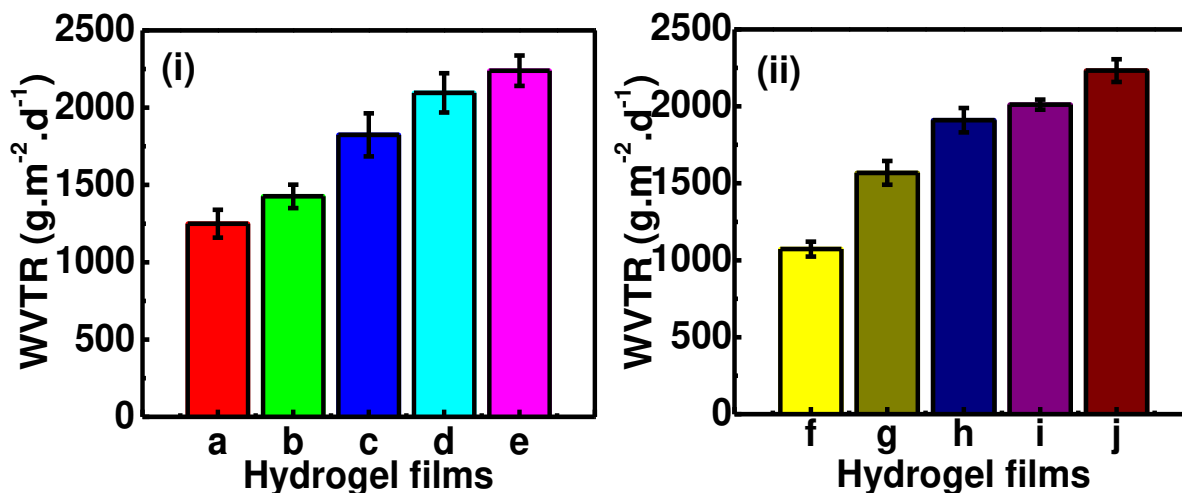


Figure 4. Water vapour transmission rate (WVTR) of (i) 3.5% PVA+ 0.5% gelatin (Control) and its other combinations with 0.5-2.0% AgNPs(a-e) hydrogel films respectively; (ii) 3.5% PVA + 1.0% gelatin(Control) and its combinations with 0.5-2.0% AgNPs(f-j) hydrogel films respectively.

2.6. Study of Hydrophilicity and Wettability by Contact Angle Measurement

Hydrophilicity and wettability analysis of biomaterials is a significant study in biomedical applications as they come in contact with the blood and exudates. So water contact angle measurements are used to study the membrane surface hydrophilicity[26]. An ideal wound dressing material generally should have a contact angle $< 90^\circ$ with a good ability to absorb excessive exudates from the wound sites[24]. Keeping a view on this, we studied hydrophilicity and wettability by contact angle measurement. Thus, **figure 5(i)** and **(ii)** represent the contact angle measurements and images of the various PVA-gelatin-AgNPs hydrogel film formulations. Therefore, we observed that the addition of AgNPs greatly influences the contact angle in both the combinations of PVA-gelatin hydrogel films. The experiment was performed with a constant concentration of PVA while the gelatin concentration was varied and AgNPs concentration was gradually increased. The contact angles of the AgNP incorporated PVA-gelatin hydrogel films are

comparatively similar to that of the control PVA-gelatin hydrogel blend with no AgNPs. From **figure 5(i)**, it is clear that there was a slight decrease in the contact angle with no further substantial decline initially. This may be due to AgNPs aggregation in the hydrogel at higher concentrations[26]. However, **figure 5(ii)** showed no considerable decrease in contact angle after adding AgNPs into the polymer hydrogel films. We observed the contact angle ranges in-between 55.37 - 85.33° showing good ability to absorb water or exudates.

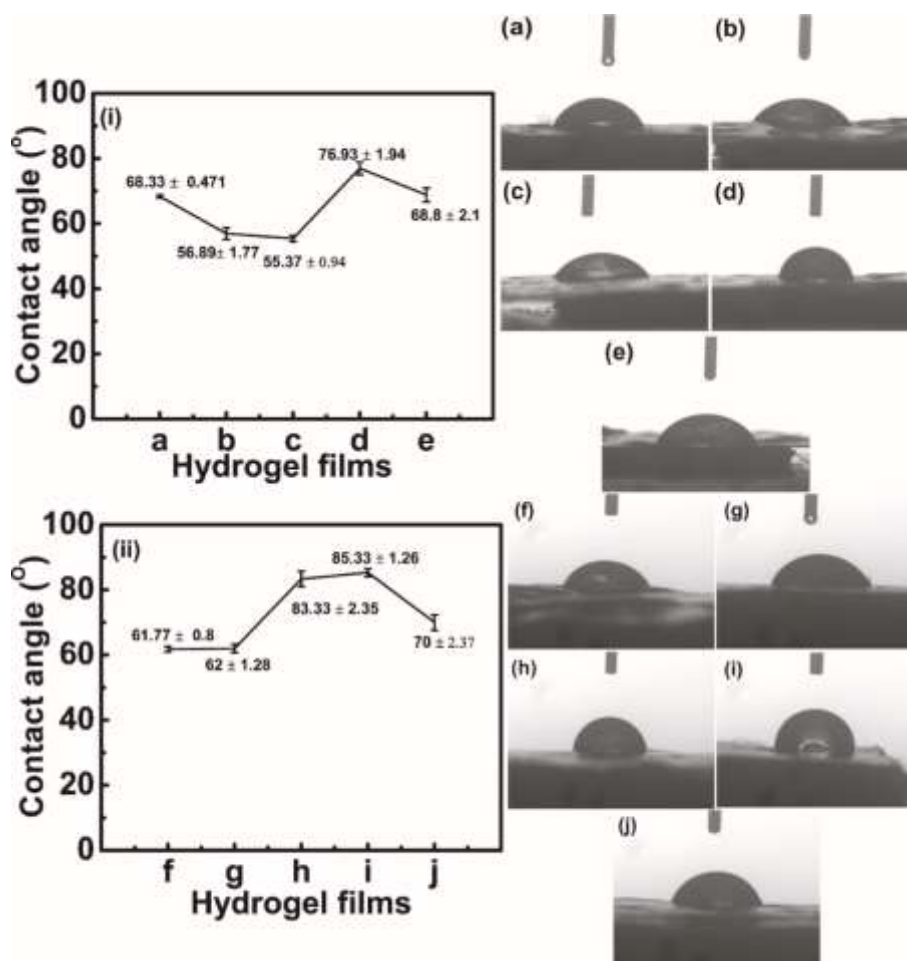


Figure 5. (i) Graphical representation of the contact angles of (i) 3.5% PVA+0.5% gelatin+ AgNP (with varying concentration) and (ii) 3.5% PVA+1.0% gelatin+AgNPs (with varying concentration) hydrogel film formulations along with their contact angle measurement images.

2.7. Study of Morphology of NC Hydrogel Films by FESEM

Next, we studied the surface morphology of the hydrogels by the FESEM technique. The FESEM micrographs of PVA-gelatin hydrogel films and PVA-gelatin-AgNP hydrogel films are shown in **figure 6 (i-viii)**. From **figure 6(i)**, **(ii)**, **(v)**, and **(vi)**, the microstructure of the control polymeric hydrogel film is analyzed. Thus, the surface of the polymeric PVA-gelatin hydrogel, i.e., 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0% gelatin blend without AgNPs, showed clear or plain surface without much irregularities confirming the homogeneity of mixed PVA-gelatin polymer hydrogel blends. The hydrogels are transparent and mostly amorphous. **Figure 6(iii)** and **(iv)** displays FESM of 3.5% PVA + 0.5% gelatin + 2% AgNP hydrogel films whereas **figure 6(vii)** and **(viii)** displays for 3.5% PVA + 1.0% gelatin + 2% AgNP hydrogel films. After adding 2% AgNPs in both the combinations, the structure changed to be rough but showed a homogenous surface of the polymeric hydrogel film matrix with silver nanoparticles distributed evenly[27]. These micrographs confirm the incorporation of AgNPs with dimensions smaller than 100 nm within the polymeric matrix. At higher concentrations of AgNPs, aggregations were observed in the polymeric hydrogel matrix as the average particle size of polydisperse AgNPs originally synthesized using Bhimkol (*Musa balbisiana*) peel extract and which were embedded into the PVA-gelatin hydrogel film was 44 nm[15].

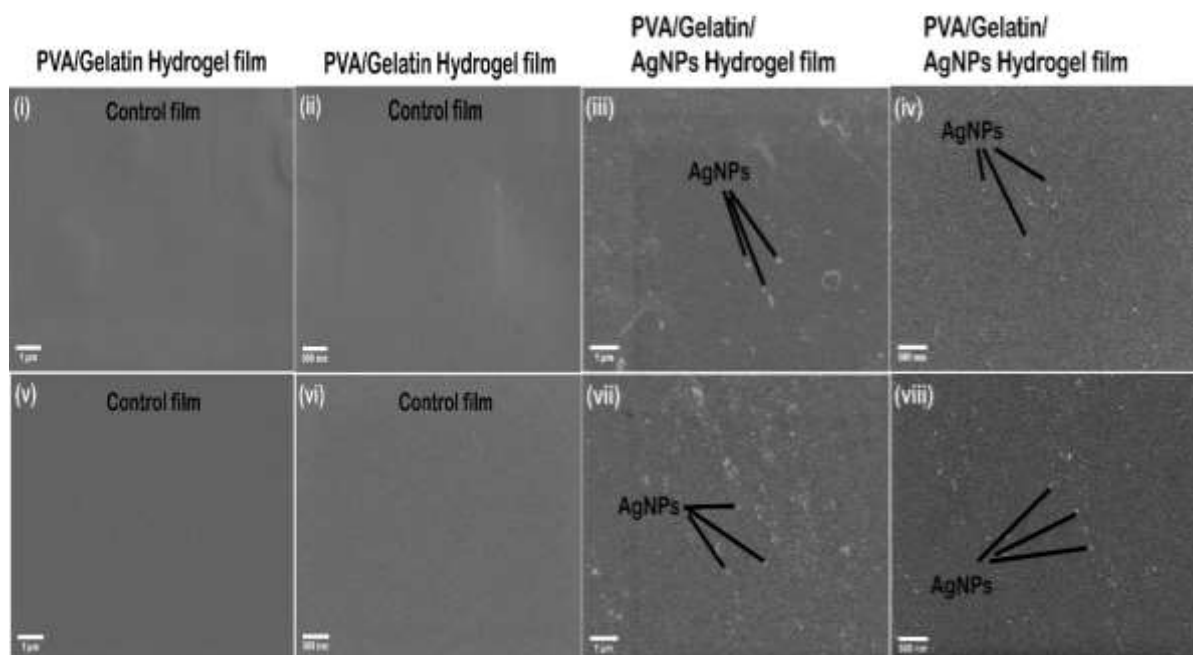


Figure 6. (i-iv) represents FESEM images of 3.5% PVA+0.5% gelatin hydrogel films and 3.5%PVA+0.5% Gelatin+ 2% AgNPs hydrogel films at 1 μ m scale (25kx magnification) and 500 nm scale(50kx magnification), (v-viii) represents FESEM images of 3.5% PVA+1.0% gelatin hydrogel films and 3.5%PVA+ 1.0% gelatin+ 2% AgNPs hydrogel films at 1 μ m scale(25kx magnification) and 500 nm scale(50kx magnification) respectively.

2.8.Study of Morphology of NC Hydrogel Films by Field Emission Transmission Electron Microscopy (FETEM) Analysis

Next, the surface morphology of the PVA-gelatin hydrogel blend was studied by the FETEM analysis. The SAED pattern determines the amorphous or crystalline nature of the polymeric hydrogel film. The existence and distribution of AgNPs within the polymer matrices in the triad polymeric NC-hydrogel films was also investigated by FETEM analysis. **Figure 7** represents the FETEM images of PVA-gelatin polymeric hydrogels (control) and PVA-gelatin-AgNPs NC-polymeric hydrogels providing the size and shape of the AgNPs, which were observed in the polymeric hydrogels.

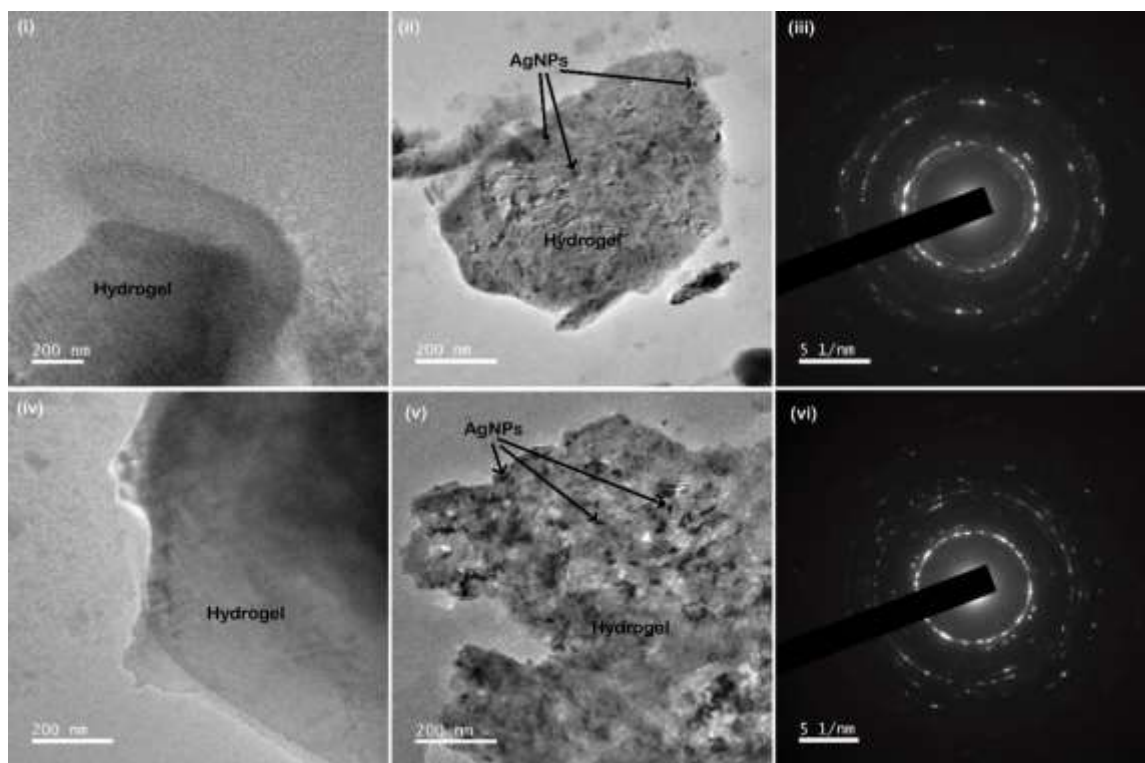


Figure 7. FETEM images of (i) 3.5% PVA + 0.5% gelatin hydrogel film(control), (ii) 3.5% PVA + 0.5% gelatin + 2% AgNPs, (iii) SAED pattern of the 3.5% PVA + 0.5% gelatin + 2% AgNPs hydrogel film. (iv) FETEM images of 3.5% PVA + 1.0% gelatin hydrogel film(control), (v) 3.5% PVA + 1.0% gelatin + 2% AgNPs and (vi) SAED pattern of 3.5% PVA + 1.0% gelatin + 2% AgNP hydrogel film.

Thus, figures 7(i) and (iv) showed the hydrogel matrix formation between PVA and gelatin without many irregularities. Both the control hydrogel films i.e., 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0% gelatin are smooth. Again, **figure 7(ii)** and **(v)** showed the FETEM pattern of NC-hydrogel films, 3.5% PVA + 0.5% gelatin + 2%AgNPs and 3.5% PVA + 1.0% gelatin + 2%AgNPs which clearly revealed the presence of AgNPs. **Figure 7(iii)** and **(vi)** represents the SAED pattern of 3.5% PVA + 0.5% gelatin + 2%AgNPs and 3.5% PVA + 1.0% gelatin + 2%AgNPs hydrogel films. Thus, we observed that the AgNPs are almost polydispersed with

mostly spherical. Further, the SAED pattern also shows the random distribution of AgNPs within the polymeric matrices of PVA-gelatin blends. We also observed that the AgNPs had an average particle size of around 12-66 nm when analyzed using ImageJ software. **Figures 7(ii) and (v)** showed the nanoparticle agglomeration due to the addition of a high concentration of AgNPs(2% v/v) and thus, making it difficult to measure the exact particle size and separate the AgNPs[28]. However, we could observe the nanoparticles integrated within the polymer hydrogel matrix and scattered smaller-sized AgNPs. This may be because some nanoparticles might have come out of the swollen hydrogel matrix during sonication in hydrogel preparation. The AgNPs have face cubic structure and in the SAED pattern, the pattern was studied of the triad polymeric NC hydrogel film, which indicated some crystallinity to the hydrogel film[26]. So, it can be concluded that in situ AgNP formation within a well-defined crosslinking hydrogel network matrix can produce controlled and smaller-sized nanoparticles. Further, the presence of elemental silver was confirmed by energy-dispersive X-ray analysis (**Figure 8**).

2.9.Study of Elemental Analysis of the NC Hydrogel via EDX

The presence of AgNPs in the NC-hydrogel films was confirmed by the EDX analysis conducted on the PVA-gelatin nanocomposite hydrogel films. **Figure 8** represents the elemental distribution of the PVA-gelatin-AgNP hydrogel films. While **figure 8(i) and (ii)** represent EDX showing C, O and Ag, of the control (without AgNPs) PVA-Gelatin polymer blend hydrogel films [i.e., 3.5% PVA + 0.5% Gelatin and 3.5% PVA + 1.0% Gelatin], **figure 8(ii) and (iv)** represent the same for PVA-Gelatin hydrogel films containing 2% AgNPs [i.e., 3.5% PVA + 0.5% Gelatin + 2% AgNPs and 3.5% PVA + 1.0% Gelatin + 2% AgNPs]. For mounting the hydrogel films, carbon tape was used, it justifies the presence of carbon in the elemental analysis. The presence of Ag in the composite hydrogel films was observed from EDX analysis.

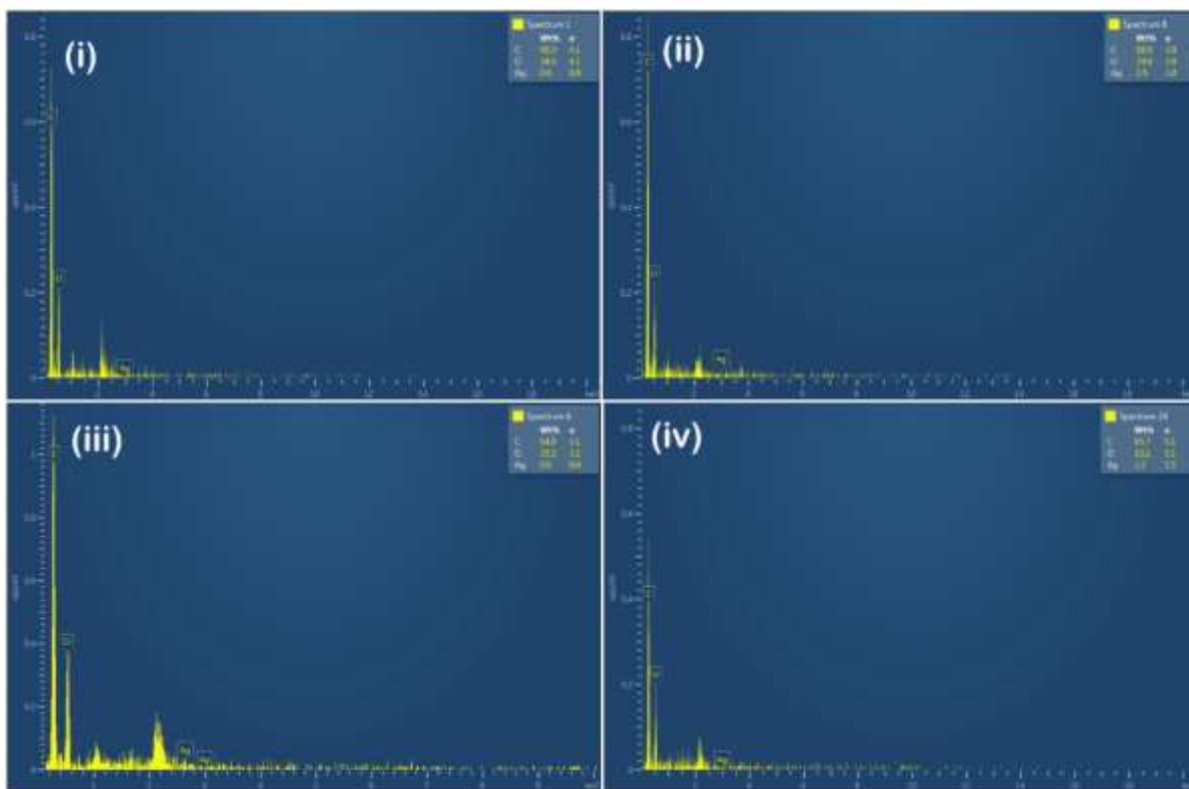


Figure 8. EDX analysis of the PVA/Gelatin hydrogel films (i) 3.5% PVA+ 0.5% gelatin (ii) 3.5% PVA + 0.5% gelatin + 2.0% AgNP, (iii) 3.5% PVA+ 1.0% gelatin and (iv) 3.5% PVA + 1.0%gelatin+ 2.0% AgNP

2.10. Study of X-Ray Diffraction of the NC Hydrogel

The microstructures of the PVA-Gelatin hydrogel film with and without incorporation of AgNPs were investigated by the wide-angle X-Ray Diffraction. The X-ray diffraction spectrum for the control PVA-gelatin hydrogel films and PVA-gelatin-AgNPs hydrogel films are shown in **figure 9**. The XRD pattern of both PVA-gelatin hydrogel film without AgNP and with 2% AgNP showed a sharp and prominent 2θ peak at around 19.6° with very high intensities of 5670 and 4858, respectively. This peak indicates that the crystallinity of the hydrogel films is mainly influenced by gelatin more than PVA[10]. Also, it specifies the semi-crystalline nature of PVA, which comprises both amorphous and crystalline regions. Small crystallites are distributed arbitrarily in the amorphous matrix considered the crystalline phase[29]. Furthermore, a much

broader hump was observed at 2θ of 40° with much lower intensities for both the hydrogel films. This peak confirms the presence of PVA in the composite hydrogels.

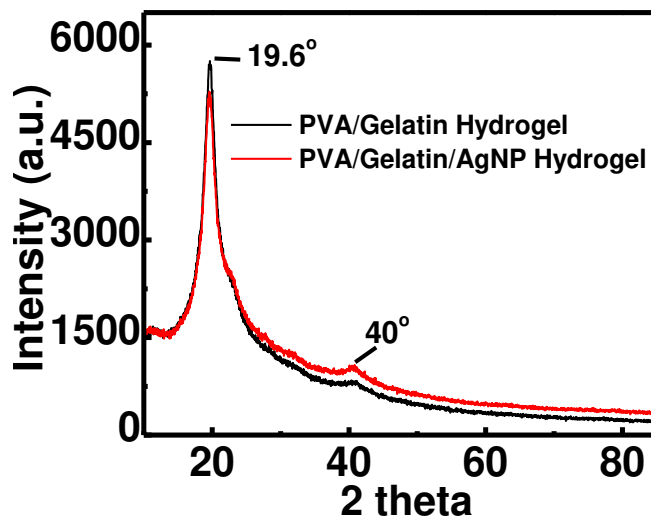


Figure 9. X-Ray Diffraction patterns of PVA-Gelatin hydrogel and PVA-gelatin-AgNP hydrogel films.

Next, we calculated the d-spacing and crystallinity percentages from the 2θ values of the X-Ray diffraction pattern. Thus, we observed that percentage crystallinity decreases from 79.87% to 72.02% after adding AgNPs into the polymer hydrogel (**Table 4**). This is possibly due to the interaction of Ag ions with the polymer chain, mainly with the hydroxyl groups present in the hydrogel matrix.

Table 4. Crystalline sizes and percentage crystallinity of the hydrogel films.

Hydrogel samples	Crystalline size(d)	Crystallinity
	nm	(%)
PVA/Gelatin Hydrogel	2.16	79.87
PVA/Gelatin/AgNP Hydrogel	0.27	72.02

2.11. Study of Thermal Property of NC Hydrogel Films

The thermal stability property is critical in materials for application in biomedical and packaging fields. Thus, the thermal analysis of the PVA-gelatin polymeric hydrogel film and the PVA-gelatin NC hydrogel films were investigated using a Differential scanning calorimeter (DSC/TGA Analyser). The dry hydrogel films were evaluated in a temperature range of 20-600°C in a TGA analyser and the thermal stabilities of these hydrogels were recorded based on the weight change. **Figure 10 (i)** and **(ii)** represent the TGA and DSC thermograms for the PVA-gelatin hydrogel film and PVA-gelatin-AgNPs hydrogel films, respectively. Thus, the TGA thermogram exhibited two main decomposition steps **Figure 10(i)**. Among them, the initial decomposition temperature was from 100°C to 200°C, wherein we found that both the hydrogels exhibited slight weight loss (3-6%) initially that may be due to water molecule evaporation. The PVA-gelatin polymer blend hydrogel showed less weight loss at this temperature which started at 150°C. This may be due to the hydrophobic nature of PVA, which underwent partial bond scission. However, at the latter stage, the blend hydrogel recovered at a higher temperature up to 200 °C, possibly by a reformation of bonds.

On the other hand, the TGA curve of the PVA-Gelatin-AgNP hydrogel shifted towards a higher temperature than the PVA-gelatin polymer hydrogel blend (control). The degradation was found at higher temperatures (270°C) due to the intermolecular disintegration and partial breaking of

molecular structure. Again, above 380°C, the PVA-gelatin polymeric hydrogel blend (control) was found to decompose completely. In contrast, the silver nanoparticle incorporated hydrogel film (PVA-Gelatin-AgNP hydrogel) showed better thermal stability. The weight loss above 480°C was observed, which indicated the delay of the thermal degradation process. Above 500 °C, the residual mass was around 8.98% and 9.95% for PVA-gelatin hydrogel and PVA-gelatin-AgNP hydrogel films, corresponding to carbon weight and ash after decomposition.

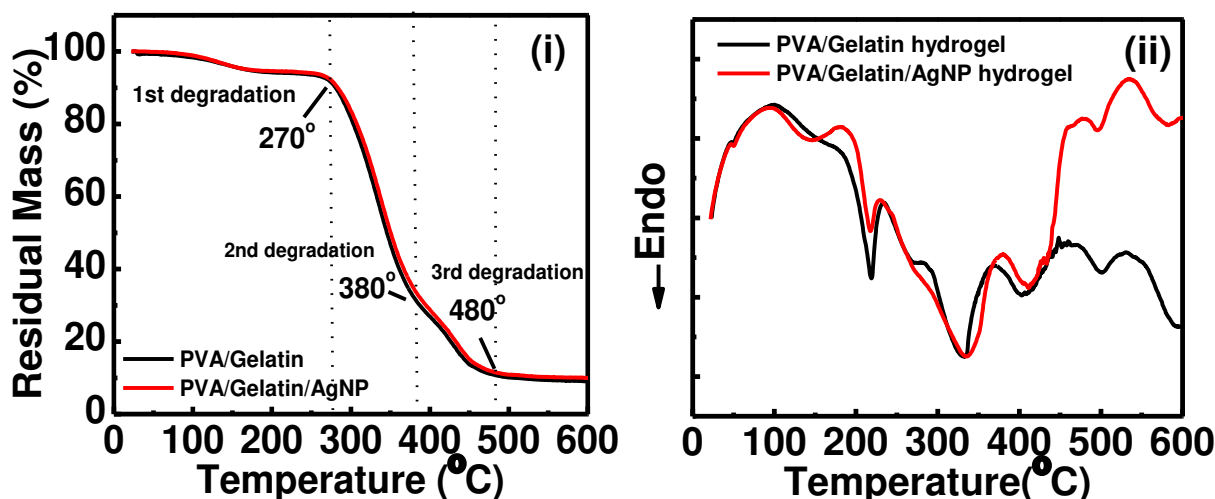


Figure 10. (i) Thermal Gravimetric Analysis (TGA) curves of the PVA-Gelatin hydrogel film and silver nanoparticle incorporated PVA-Gelatin hydrogel films. (ii) DSC thermograms for the PVA-Gelatin hydrogel film and silver nanoparticle incorporated PVA-Gelatin hydrogel films.

In the DSC thermograms shown in **figure 10 (ii)**, the endothermic peaks corresponding to the melting point of PVA was obtained at around 200-230°C. Also, the endothermic peaks corresponding to the glass transition of gelatin were observed in the same range of the melting temperature of PVA. As such, the endothermic peaks of PVA-Gelatin hydrogels are considered as the melting temperature of PVA. There are two significant endothermic peaks at around 220°C, which is the melting transition peak, and at 330°C, which indicates the degradation temperature of PVA. On the other hand, the melting transition peaks for PVA-Gelatin-AgNPs NC-hydrogel films were the intermediate value of the two polymers (PVA and Gelatin). The transition width of the

AgNPs incorporated polymeric hydrogels was similar to the PVA and gelatin components. Therefore, the incorporation of AgNPs has little significance in the peak shifting, indicating higher stability of the PVA-Gelatin hydrogel films. The addition of AgNPs in the PVA-Gelatin hydrogel lowers the melting temperature slightly, which may be because the gelatin acts as an impurity in lowering the melting temperature of the hydrogels. The degradation temperature also changes due to the incorporation of AgNPs into the polymeric matrices. The miscibility of both the polymers with AgNPs might be the reason for the depression of the melting temperature and the degradation temperature changes. The decrease of the melting temperature interprets the interaction of the PVA, Gelatin and AgNPs, causing strong compatibility among the components and resulting in a better polymeric hydrogel nanocomposite material[20,29].

2.12. Study of Antibacterial Activity of NC Hydrogel Films

The antibacterial activity of the NC polymer hydrogel films developed using PVA, Gelatin and AgNPs were next evaluated by the zone of inhibition study using the disc diffusion method. *E. coli* (gram-negative) and *S. aureus* (gram-positive) are the most common bacteria causing severe infection on the skin and other soft tissues, delaying the wound healing process. The zone of inhibitions caused by the hydrogel films against *E. coli* and *S. aureus* after 12 hours are shown in **figure 11(A)**. Two combinations of the polymer hydrogels films i.e., 3.5% PVA + 0.5% gelatin and 3.5% PVA + 1.0 % gelatin and their nanocomposites were studied. In both cases, the control PVA-Gelatin polymer blend hydrogel films (without AgNPs) showed no zone of inhibition against both *E. coli* and *S. aureus* indicating no antibacterial activity exhibited by the control hydrogel films. On the other hand, AgNPs loaded hydrogel films showed potent antibacterial activity against both *E. coli* and *S. aureus* except for hydrogel films with 0.5% AgNP. PVA-gelatin hydrogel films with 2% AgNPs showed the highest zone of inhibition against both *E.coli* and *S.aureus* among all

the other combinations tested. The NC-hydrogel film with 3.5% PVA + 0.5% gelatin + 2% AgNP showed the highest zone of inhibition, indicating a potent antibacterial activity against *E. coli* and *S. aureus*. The antibacterial activity is mostly due to the Ag⁺ ions released from silver (Ag) particle oxidation in the hydrogel, causing bacterial cell division and bacterial respiratory chain damage after moisture contact, ultimately leading to bacterial cell death[30]. Thus, PVA-gelatin hydrogel with 2% AgNP would be effective for wound dressings. Further, the antibacterial activity of 3.5% PVA + 0.5% Gelatin + 2% AgNP hydrogel film was more than 70 % against both *E. coli* and *S. aureus* bacteria, as shown in **figure 11 (B)**.

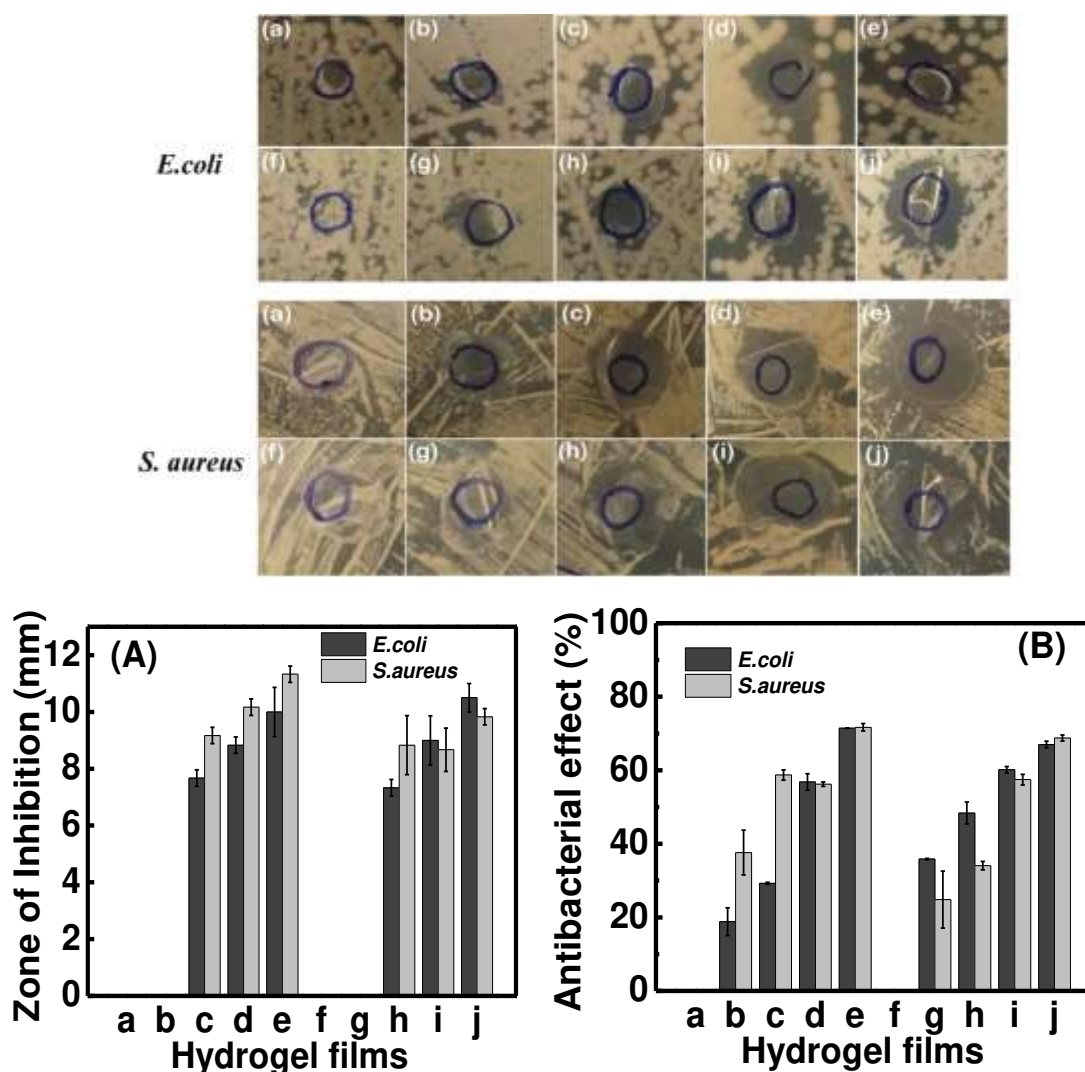


Figure 11. Antibacterial activity of the PVA/Gelatin/AgNP hydrogel films (a-j) against *E. coli* and *S. aureus*. (A) Zone of inhibition diameters (mm) of all the combinations (a-j) and (B) Antibacterial effect (%) of the hydrogel films (a-j). Film no. (e) 3.5% PVA + 0.5% Gelatin + 2% AgNP hydrogel film showed >70 % of antibacterial effect against both *E. coli* and *S. aureus* bacteria.

2.13. Study of Cell Viability of NC Hydrogel Films

After observing good antibacterial activity, we next test the effectiveness of the films to be utilized for wound healing purposes. Thus, we studied the biocompatibility of the films using BJ human foreskin fibroblast cells. For that purpose, the morphology of the BJ human foreskin

fibroblast cells was first examined, which were subjected to exposure with the control polymer hydrogel film and AgNP incorporated hydrogel films. As shown in **Figure 12A**, no significant changes in the morphology of BJ fibroblasts were observed on exposure to these films. Next, the effect on the viability of human cells with the hydrogel films was analyzed using MTT assay. The absorbance was measured at 540 nm, and then a bar graph was plotted (n=3) (**Figure 12B**). The BJ fibroblasts were thus exposed with PVA-Gelatin hydrogel film and PVA-Gelatin-AgNP hydrogel film for 24 and 48 hours. Relative to untreated BJ cells, the viability of BJ cells with PVA-Gelatin hydrogel and PVA-Gelatin-AgNP hydrogel films was calculated. Thus, a significant difference in the viability of BJ cells was observed after 48 hours of treatment with AgNP incorporated hydrogel film (cell viability >67%) compared to that of the PVA-Gelatin polymer blend hydrogel film (control, cell viability >59% keeping untreated BJ cells as 100%). This result further confirmed that the AgNP incorporated hydrogel film is non-toxic and does not affect the viability of human cells. Thus, the MTT assay demonstrated the biocompatibility of the AgNP incorporated PVA-Gelatin hydrogel film, which can be used for various biological applications[20].

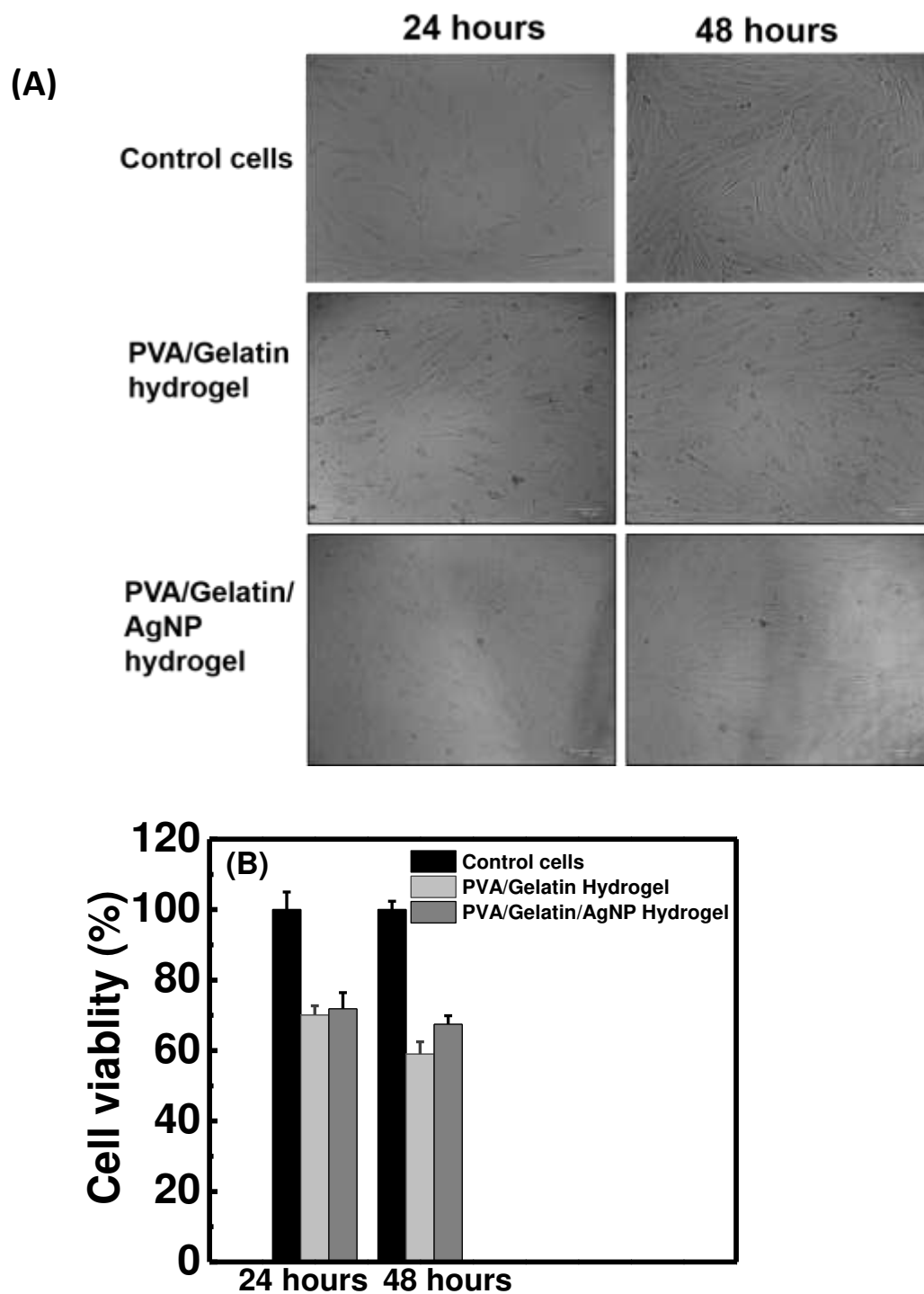


Figure 12. (A) Morphology of the BJ fibroblast cells on exposure with the polymer hydrogel films at 24 and 48 hours using an inverted brightfield microscope at 20x magnification. (B) Percentage cell viability of PVA/Gelatin and PVA/Gelatin/AgNP hydrogel film after 24 and 48 hours (keeping control sample at 100%).

2.14. Study of Wound Healing Property of NC Hydrogel Films via In vitro Scratch Wound

Healing Analysis

Next, the wound healing property of the biocompatible PVA-Gelatin-AgNP hydrogel film on a monolayer of BJ fibroblast cells was studied by the *in vitro* scratch assay. For this analysis, BJ human foreskin fibroblast cells were used due to their crucial role in cell migration, one of the essential characteristics of scar formation and skin wound healing[31–33]. Further, the synthesis of extracellular matrix and different growth factors contributes to wound healing[34]. As such, BJ fibroblast cells were used to evaluate the wound closure effects of the PVA-Gelatin-AgNP hydrogel film using *in vitro* scratch assay method. Migration of BJ cells was observed within 6 hours of exposure. The cells migrated towards the center of the wound area with the PVA-Gelatin-AgNP hydrogel film. In contrast, no migration occurred in the cells with the control PVA-Gelatin hydrogel film even after 6 hours of exposure (**Figure 13A**). More than 90% of wound closure was observed for both NC hydrogel films after 24 hours of exposure. However, we observed a significant difference in the rate of wound closure at 6 and 12 hours between PVA-Gelatin-AgNP hydrogel film and the PVA-Gelatin control hydrogel film. The wound closure (%) was calculated using Image J software, and a bar graph was plotted (n=2) and shown in **figure 13B**. The results signify that PVA-Gelatin-AgNP NC hydrogel film has more efficient wound healing potential than their control PVA-Gelatin polymer blend hydrogels (**figure 13B**).

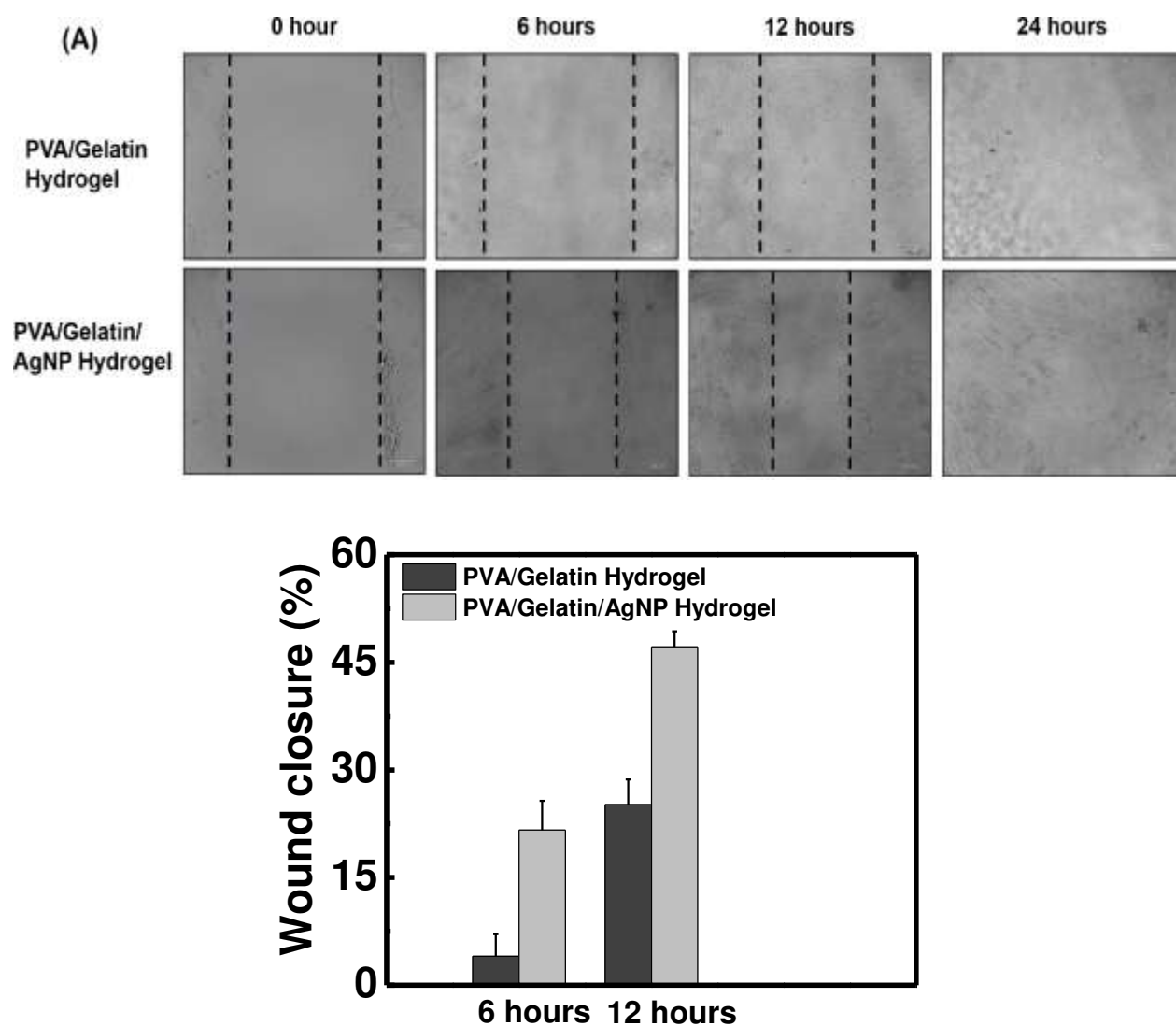


Figure 13. (A) Illustrative micrographs of the human cells migrating into a scratch area over 24 hours in PVA-Gelatin (control) and PVA-Gelatin-AgNP hydrogel. (B) The percentage wound closure or the migration rate of the cells in PVA-Gelatin and PVA-Gelatin-AgNP hydrogel films.

3. CONCLUSION

Nanocomposite hydrogel was developed by blending polyvinyl alcohol (PVA), gelatin biomacromolecule and silver nanoparticles (AgNPs). Incorporation of silver nanoparticles (AgNPs) into the polymer matrix resulted in a potential functional wound dressing material. AgNPs, with gelatin and PVA blend, have facilitated the hydrogel films development due to their non-toxic and biocompatible nature. The PVA-Gelatin hydrogels have superior crosslinking within their matrices. The prepared nanocomposite polymer hydrogel films observed better swelling behaviour, mechanical strength, and thermal stability.

The cell viability was more than 90% after 24 and 48 hours for the PVA-Gelatin-AgNP hydrogel, which means the nanocomposite hydrogels showed no toxicity. The antibacterial effect was >70% for the PVA-Gelatin hydrogel with 2% AgNPs against *E. coli* and *S. aureus*, which indicated that the higher the concentration of AgNPs incorporated, the antibacterial efficiency increases in the hydrogels. Wound closure was more than 90% after 24 hours of exposure in both control and AgNPs contained hydrogel films when evaluated by the scratch assay on human foreskin cells. PVA-Gelatin-AgNP hydrogel film has a faster cell migration rate than PVA-Gelatin control hydrogel film. Thus, the PVA-Gelatin-AgNPs hydrogel film accelerates the wound healing procedure and potentially protects the wounded skin surface against exudate accumulation and dehydration. These also prevent bacterial growth and infection in the injured area. We are currently developing dressing materials utilizing such films for wound dressing purposes that could address chronic wound healing.

4. EXPERIMENTAL SECTION

4.1. Materials and Methods

Poly (vinyl) alcohol i.e., PVA powder was purchased from LOBA Chemicals, India. Its molecular weight is 111,000 g/mol. Gelatin purchased from SRL Pvt. Ltd, India, which could be used without any further purification. Silver nitrate(AgNO_3) was purchased from Sigma-Aldrich, India. Milli-Q water was used for all the experimental works. Other chemicals, equipment and glassware used were hot plate, magnetic stirrer, magnetic bead, weighing machine, glass beakers, hot air oven, petri dishes, marker pen, acetone, mortar and pestle, hydraulic press, Laminar hood (for antimicrobial works), Bunsen burner, sterile L-spreader, ethanol, tissue paper, incubator, pipette, Eppendorf tube, tweezer, scissor, centrifuge, falcon tubes, micropipette tips, copper-coated TEM grid, carbon tape.

4.2. Green Synthesis of AgNPs

AgNPs were synthesized using Bhimkol (*Musa balbisiana*) peel extract via the green route with slight modification[35]. Biological reduction of AgNO_3 was carried out initially: To optimize the synthesis route for developing silver nanoparticles (AgNPs), the reaction solution concentrations 1-50% v:v ratio of Banana peel extract: AgNO_3 Solution were varied. The reaction mixture was kept in the dark at room temperature to avoid any photoactivation of silver nitrate under inert conditions. The yellowish-brown color was observed after 30 mins indicating the formation of silver nanoparticles. Differential parameters like variation in AgNO_3 concentration, time, and pH were reported to optimize AgNPs synthesis[15].

4.3. Preparation of the Polymer Hydrogel Nanocomposites

Poly (vinyl) alcohol (PVA) and PVA-Gelatin hydrogel films with different concentrations of PVA and gelatin were prepared (% w/w ratio). The solution casting method was used to prepare PVA-Gelatin films' which is already described above[6].

4.4. Study of Swelling Kinetics

The dry hydrogel films were submerged in deionized water and incubated in a water bath at 37°C; then the hydrogel was taken out at regular intervals (after every 30 mins). The hydrogel was dabbed in a tissue cloth to remove excess surface water and then wet hydrogels were weighed until equilibrium was reached.

The water uptake capacity (Wu) was determined by the formula given below:

$$Wu = \frac{Mt - Mg}{Mg} \times 100\%$$

Here, Wu represents the water uptake capacity.

Mt is the weight of the hydrogel at regular time intervals

Mg is the initial weight of the hydrogel.

The water-holding performance of these hydrogels was determined by placing the swollen hydrogels at room temperature until they attained a constant weight. Then the hydrogels were weighed at regular intervals of time[20].

4.5. Study of Mechanical Properties

Tensile strength of the polymer hydrogel films (control) and the green synthesized AgNPs incorporated polymer hydrogels films were measured by tensile test using a 5kN Electromechanical Universal Testing Machine (UTM). Tensile test was performed on Zwick

Roell: Z005TN UTM. The size of the specimen was prepared according to ASTM standards. The hydrogel films were cut into 10 mm×60 mm×1 mm (Width × Height × Thickness) pieces and placed under 5kN load cell; the stretching speed was kept at 10 mm/min at room temperature to measure the tensile strength and the elongation at break.

4.6. Study of FT-IR Spectroscopy

Fourier Transform Infrared (FT-IR) spectroscopy was verified by ATR-FTIR spectrophotometer in the range of 400-4000 cm^{-1} wavenumbers and a resolution of 4 cm^{-1} for the PVA/Gelatin/AgNPs hydrogels. The transmittance mode was used for the spectrum. FTIR spectroscopy characterizes the occurrence of specific functional chemical groups and their interactions. PVA-gelatin cross-linking is easily detected by FTIR analysis.

4.7. Water Vapour Transmission Rate(WVTR) Analysis

The hydrogel films' water vapour transmission rate(WVTR) was measured using the ASTM E 96-95 standard method. Measuring dishes that were corrosion-resistant, airtight and water-resistant was taken. The gap between the liquid level(water) and the specimen (hydrogel films) was about 5 ± 1 mm. The hydrogel film samples were cut into square pieces with a thickness of 1.5 mm approx. and sealed over the opening of dishes making square cut of 20 mm×20 mm in the aluminum foils used to cover the dishes. The dishes were filled with 20 mL of distilled water each. The hydrogel films were fastened tightly using tape with aluminum foil to prevent any moisture loss from the sides of the hydrogel films. The initial total weight of the dish, hydrogel film, distilled water was weighed and recorded as W1. This experiment was carried out in an incubator kept at 37°C. After 18 hours and 24 hours intervals, the W2 (final weight) was recorded. The water vapour transmission rate(WVTR) is also called moisture vapour transmission rate(MVTR); it is calculated by the following formula:

$$WVTR = \frac{W1 - W2}{T} \times 1000 \times 24$$

Where,

WVTR represents water vapour transmission rate,

W1 represents the initial weight of the dish, hydrogel film and water(g),

W2 represents the final weight of the dish, hydrogel film and water(g) and

T represents the time (hours)[20,36].

4.8. Contact Angle Measurements Analysis

Quantitative measurement of wetting of a solid by a liquid is referred to as the contact angle (θ , theta) measurement. The angle formed at the three-phase boundary where a liquid, gas, or a solid interconnects by the liquid is the contact angle. Wound dressing material of surface having $<90^\circ$ of contact angle is considered ideal as it has a good ability to absorb the wound exudates[23]. Contact angle was measured using a drop shape analyser by Kruss Company, Germany. The hydrogel films were cut into 10×10 mm pieces and placed on the sample holder of the contact angle measurement apparatus. A small drop of distilled water $<5 \mu\text{L}$ was injected into the hydrogel surface using a micro-syringe. The contact angle was measured at room temperature and three replicates were measured for each hydrogel composition[24,27].

4.9. Antibacterial Properties

The disc diffusion method evaluated the antibacterial activity of the polymer nanocomposite hydrogels. A gram-negative bacteria, *E.coli* (1969) and gram-positive bacteria, *S.aureus* (ATCC33592) were used in this experiment, respectively. $100 \mu\text{L}$ of the bacterial (*E.coli* and *S.aureus*) suspension (10^8 CFU/mL) was spread on LB(Luria-Bertini) agar plates. The hydrogel film samples were evenly punched into circular discs of 5 mm diameter and placed on the surface

of the LB agar plates. Then the plates were incubated at 37°C for 12 hours, zone of inhibition diameter was measured[30]. 100 mg of the hydrogel films were added to 2 ml of bacterial culture suspensions (*E. coli* and *S. aureus*) each and incubated for 12 hours at 37°C in a shaker incubator at 180 rpm. For all the hydrogel samples, measurement of optical density (O.D) and CFU/mL count took place at 600 nm. The antibacterial effect was evaluated by using the following equation:

$$\% \text{ AE} = ((C-H)/C) \times 100$$

AE is the antibacterial effect, C is the number of bacteria on the surface of the control sample, and H is the number of bacteria on the surface of the hydrogel film samples[24].

4.10. FESEM Analysis

Field Emission Scanning Electron Microscopy (FESEM) can be used to obtain the surface morphology of the prepared polymeric hydrogel nanocomposite films in higher resolution of imaging. Gemini 300 FESEM from CARL ZEISS company was used for both the FESEM and EDX analysis. The FESEM imaging was carried out at a resolution of 200 nm and 1 µm scale at 3-4 kV accelerating voltage and at 25 kx - 50kx magnification. The elemental analysis of the hydrogels was also conducted using energy-dispersive X-ray spectroscopy. Both the qualitative and quantitative analysis were performed.

4.11. FETEM Analysis

The Field Emission Transmission Electron Microscopy (FETEM-JEOL 2100F) technique is used for higher resolution imaging of the PVA/gelatin/Ag nanoparticles hydrogel complex. Morphology, size and shape of the nanoparticles, and the polymer blend formation can be easily determined.

4.12. Thermogravimetric(TG) Analysis

The thermal analysis was performed using a differential scanning calorimeter DSC/TGA analyzer (NETZSCH, STA449F3A00). Around 5.8 mg of the hydrogel film sample was placed into the ceramic crucible and the heating rate was kept at 10°C/min under Argon atmosphere in a temperature range of 20°C-600°C. The thermogravimetric analyser was mainly used for analyzing the chemical structure of the polymer hydrogel film samples[20].

4.13. X-Ray Diffraction Analysis

X-Ray Diffraction of the hydrogel films was recorded by using Bruker D8 Advance X-ray diffraction meter (Netherlands) with “Ni-filtered” Cu anode K α radiation of wavelength $\lambda = 1.54060 \text{ \AA}$. At room temperature, between 10-85° range of 2θ at a scanning speed of 1°/mm with a step size of 0.05° respectively, the scan was performed. The crystalline size is calculated using the following Scherrer equation:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

D is the crystal dimensions, λ is the X-ray wavelength, k is scherrer constant(0.9 rad), β is FWHM (full width at half maximum) and θ is the bragg angle[6,24].

4.14. Cell Viability Assay

BJ normal human foreskin fibroblasts (ATCC catalog CRL-2522) were cultured in Dulbecco's Modified Eagle Medium (DMEM, GIBCO) supplemented with 10% Fetal Bovine Serum (FBS,

GIBCO), 1% non-essential amino acids (NEAA; GIBCO) and Penicillin-Streptomycin solution (GIBCO; 100 U/ml penicillin and 100 µg/ml streptomycin). The cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C in a CO₂ incubator (Eppendorf). The medium was changed every second day with complete media in all experiments.

Cell viability assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)[37]. Briefly, BJ human foreskin fibroblast cells were seeded in a 96-well flat-bottomed plate at a density of 4×10^4 cells per well and incubated for 24 hours in complete media in a CO₂ incubator. After that, cells were incubated with PVA/Gelatin(Control) and PVA/Gelatin/AgNP hydrogel film of 5 mm diameter(circular) for 24 and 48 hours. The medium was discarded after the treatment, cells were treated with MTT (0.5 mg/ml) for 2 hours at 37°C in a CO₂ incubator. Purple-colored formazan crystals were formed, which were then dissolved in dimethyl sulfoxide. The cell viability (%) was finally measured using a multi-plate reader (Multiscan GO, Thermo Scientific) at 540 nm.

4.15. *In Vitro* Scratch Wound Healing Assay

One of the most used techniques to study in vitro collective cell migration is the scratch assay, also known as wound healing assay[38]. BJ normal human foreskin cells were seeded in a 12-well plate at a density of 1×10^5 cells/well in the complete growth medium and then grown to full confluency. The fully confluent monolayers were scratched with a 20µL sterile pipette tip and the media was aspirated out, followed by a rinse with PBS. The scratched monolayers were incubated in standard cell culture conditions with circular PVA/Gelatin(control) and PVA/Gelatin/AgNP hydrogel films (about 17 mm diameter) in a complete growth medium for 24 hours. The images were captured at different time intervals using an inverted brightfield microscope (ZOE

Fluorescent Cell Imager, Bio-Rad) at 20x magnification. The migration efficiency was analyzed using ImageJ (1.48v) software.

4.16. Statistical Analysis

Microsoft Excel 2016 database was used for data collection and Origin 8.5 software was used for the data analysis and graph preparation. All the experiments were carried out in triplicate and the results are expressed as mean $\pm$ standard deviation (SD). The two-way ANOVA was applied to determine whether the results have significant variations and a P-value ≤ 0.05 was considered acceptable or statistically significant.

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Author Contributions

All authors have contributed equally in this work.

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Data Availability

All the data and material used in this study are available with the corresponding author and will be provided to the readers on proper requisition.

Declarations

Ethical Approval: Not applicable

Consent to Participate: Not applicable

Consent for Publication: All the authors give their consent for the publication of this research work.

Competing Interest: The authors declare no competing interests

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