Original Article

Fabrication and Characterization of Chitin Hydrogel Nano Silver Fused Scaffold for Wound Dressing Applications

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ABSTRACT

Aim/Background: A scaffold is a wound dressing material which can be fabricated in the shape of the tissue that we want to restore in our body depending upon their structural and functional requirements. Chitin is the carbon-based resource having boundless activity as a wound healing accelerator. By the addition of silver nanoparticles, the wound remedial ability and antiseptic activity of chitin can be boosted. **Materials and Methods:** Chitin was obtained from the crab shell by demineralization, heating (300°C) and dehydration. 1g, 2g, 3g, 4g and 5g of chitin was accurately weighed and formulated into five different formulations with Calcium chloride/methanol solvent. The formulated hydrogel was clarified by Whatman filter paper. Then the nanosilver solution was prepared, characterized by the pale yellow colour and added to the formulations. Further, it was lyophilized to obtain chitin hydrogel/nanosilver fused scaffold formulations namely S1, S2, S3, S4 and S5. All the five formulations were characterized for weight loss, swelling ability, porosity measurement and surface analysis. **Results and Conclusion:** The augmented formulation i.e., S5 was subjected to further characterization studies such as FT-IR analysis, optical microscopy, SEM, TEM, XRD, Zeta potential and *in-vitro* antibacterial activity. From this research, it was concluded that the chitin hydrogel nanosilver fused scaffold is a viable alternative to existing conventional dosage forms which lead to improved bioactivity and a promising biomaterial for wound dressing applications in case of administration affords to result in better patient compliance and cost-effective therapy in the field of biomedical application.

Key words: Chitin, Scaffold, Nanosilver, Hydrogel, Wound dressing.

INTRODUCTION

The wound is the disturbance of cellular and anatomic firmness of a tissue. According to the Wound Healing Society, wounds are physical injuries that result in an opening or break of skin that roots disturbance in the normal skin.[1]

A wound dressing material is the one that is applied to the wound to promote healing and to protect the wound from further harm. A wound dressing material should be designed in such a way that it should be in direct contact with the wound. The ultimate aim of the wound dressing material is to promote wound healing by providing a sterile, breathable and moist environment that facilitates granulation and epithelisation.[2]

The practice of wound dressing material with antiseptic stuff and a widerange of activity would be a good tactic for handling wound infection. An idyllic wound dressing material should hold various stuff such as that it should conserve a wet environment at the boundary of the injury place, permit movement of gases, facilitate as a bug

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barricade and get rid of surplus discharge. In addition to this, it should be harmless, non-aggravative and non-clinging; it ought to be developed from a freely available bio-stuff that necessitates nominal handling, possesses sterile chattels besides upholding wound curing.[3] Chitin is one of the most copious organic materials in nature and can be simply prepared from the shells of crab, shrimp and squid pens. Chitin and its derivatives are used for various applications in various fields. The external frame of crab entails of α-chitin and squid pen entails of β-chitin.[4]

In the medical field, it is said to possess good action as an injury healer. The wound healing capability and anti-bacterial vitality of chitin ought to be upgraded by the inclusion of silver nanoparticles. Henceforth, the chitin nano silver fused scaffolds resolve to act as a prototypical wound dressing.[5]

Thus, the present work is an effort to progress chitin hydrogel nanosilver compound scaffolds. In this study, chitin was synthesized from the crab shell followed by preparation of five formulations of chitin hydrogel. Then, nano silver solution was prepared succeeded by five formulations namely S1, S2, S3, S4 and S5 of chitin hydrogel nano silver fused scaffolds were prepared. These five formulations were initially characterized for various properties. The optimized formulation i.e. S5 was characterized by further analytical techniques.

MATERIALS AND METHODS

Materials

Crab shell was bought from the market. Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, Calcium chloride, Methanol, Trisodium citrate, Silver nitrate, ethanol and sodium citrate were purchased from Sastha Scientific Services, Chennai.

Methodology

Synthesis of Chitin from Crab Shell

The shells of the crab were cleansed and washed completely to eliminate the foreign matters. It was then followed by grinding of shells until the desired particle size was achieved. The demineralization process consists of adding the shell powder to 7% hydrochloric acid with uninterrupted stirring to avoid froth formation. In order to remove carbonate and phosphate content from the shell powder, the blend was heated at 300°C up to 3 h. To decrease the nitrogen amount of the protein, the heated mixture was treated with 5% w/v sodium hydroxide, followed by rinsing to eradicate traces of sodium hydroxide. The sample was clarified, rinsed repetitively with distilled water to get rid of chemicals and soluble impurities. The clarified sample was then dehydrated for 3 h at 700°C in an oven. The dehydrated sample of chitin was then gained. The obtained chitin was then splashed with hydrogen peroxide to lessen the colour of chitin. The above step was then succeeded by drying and stored under sealed condition.[6]

Preparation of Five Chitin Hydrogel Formulations

1 g, 2 g, 3 g, 4 g and 5 g of chitin were accurately weighed. Five litres of saturated calcium chloride/ methanol solvent was prepared and poured into five different containers such that each container has 1 litre of the solvent. Each of the weighed quantity of chitin was added into the five different containers containing the solvent. All the five containers were mixed forcefully for two days in lab temperature. Five solutions were then clarified to remove the unsolvable particles. Additional water was amassed to all the five solutions and mixed for 2 h forcefully to attain chitin hydrogel formulations. Well ahead, all the five hydrogel formulations were clarified using Whatman filter paper.[7]

Development of Nanosilver Solution

Silver nitrate solution was boiled. To this, sodium citrate solution was dripped. The solutions were stirred dynamically until a pale yellow colour was obtained. The solution was then cooled to room temperature.[8]

Fabrication of Chitin Hydrogel/ Nano Silver Fused Scaffolds Formulations

Nanosilver solution was added to each of the chitin hydrogel formulations and mixed well for 30 min. All the above mixed five formulations were lyophilized for 2 days to obtain chitin hydrogel/nanosilver fused scaffold formulations namely S1, S2, S3, S4 and S5.[9] The details regarding the fabrication of five formulations of scaffolds were given in Table 1.

Characterization of Chitin Hydrogel/ Nano Silver Fused Scaffolds Formulations

Calibration Curve of Chitin

Chitin solution was prepared with conc. HCl. 100 micrograms per ml stock solution of chitin was diluted

Table 1: Fabrication of scaffolds.

Formulation code	S1	S2	S3	S4	S5
Chitin	1 g	2 g	3 g	4 g	5 g
Saturated $CaCl_2$-CH_3OH	1 L	1 L	1 L	1 L	1 L
Silver nitrate solution	50 ml	50 ml	50 ml	50 ml	50 ml
Trisodium citrate solution	5 ml	5 ml	5 ml	5 ml	5 ml

with phosphate buffer pH 7.4. This mixture was further made up with phosphate buffer pH 7.4 to get a serial dilution of 5-25 µg/ml. Using phosphate buffer as blank, the absorbance of these solutions were measured at 390 nm.[10] The curve was constructed with absorbance (nm) against concentrations of drug (µg/ml) and the regression equation was calculated.

Weight Loss

Weight loss would be a measure of the degradation of the scaffolds with respect to time. By gestating the scaffolds in Simulated Body Fluid (SBF), the weight losses of the five scaffold formulations were conceded out at pH 7.4 and 37°C for 28 days. At different time intervals, the scaffolds were taken from the medium and dried at 50°C. The weight loss was intended by the following equation.

$$\text{Weight Loss (\%)} = [(W_o - W_t) / W_o] \times 100$$

Where, W_o denotes the weight of the fused scaffold, while W_t is the weight at time (t). The study recurred thrice and the mean values were noted.[11]

Swelling Ability

The swelling ability is a measure of the water uptake and retention properties of the scaffold. Parched weights of the scaffolds were signified as W_i. In Phosphate medium, the product was submerged for a day. It was then removed off from the medium and the wet weight (W_f) was noted.[12] The ratio of the swelling was calculated using the below equation:

$$\text{Swelling Ability (\%)} = [(W_f - W_i)/W_i] \times 100$$

Porosity Measurement

W_d was symbolized for the parched weight. After dipping in absolute alcohol for five minutes, W_l was inscribed. After slight parching over the shallow area, W_w was recorded.[12] The porosity of the scaffold was calculated using the following equation:

$$\text{Porosity (\%)} = (W_w - W_d) / (W_w - W_l) \times 100$$

Fourier Transform-Infrared (FT-IR) Analysis

The FT-IR examination helps to determine functional groups besides to know the compatibility between chitin and other excipients.[13] The spectra of the chitin and chitin hydrogel nanosilver fused scaffold (S5) were recorded sing potassium bromide pellet method in an FT-IR spectrophotometer (JASCO 4100 type A) in the range of 4000 cm^{-1} to 400 cm^{-1}.

Surface Analysis

Optical Microscopy

Optical microscopy was used to determine the surface morphology of the scaffolds using Motic Digital Microscope. The scaffold was viewed at 10X and 40X.[14]

Scanning Electron Microscopy (SEM)

In directive to ascertain the particle size and structural features, SEM was employed. The powdered sample was taken and mounted on a double sided carbon tape, which was fixed to sample specimen stub. The SEM (QUANTA FEG) instrument was used for analysis.[13]

Transmission Electron Microscopy (TEM)

TEM studies were useful in examining the morphological and crystalline arrangements of the scaffold. The principle employed to view the scaffold S5 was High-Resolution Transmission Electron Microscopy (HRTEM). The scaffold's (20 µl) solution was taken. On the carbon coated side of the copper lattice, the mixture was dripped. At room temperature for a few h, the lattice was dehydrated. The grid was then placed in the sample holder and mounted in the instrument. The instrument TECHNAI T20 was used for the analysis.[14]

X-Ray Diffraction (XRD) Analysis

XRD was employed to determine the crystal-like nature. It was performed with a PAN analytical Xpert Pro X-Ray Diffractometer. The powdered sample for evaluation was taken on the glass slide and placed on the X-Ray diffractometer.[15] The scanning rate was continued over a 2θ range of 10° to 90°.

Zeta Potential

The zeta potential provides information about the surface charge and gives insight into the interaction of biomaterials with their environment. The mean particle size of the scaffold was determined by zeta sizer NanoZS-90 (Malvern Instruments Limited). The reading was carried out at 90° angle to the incident beam of 25° using a proper dilution with filtered water (0.5 µm) filter.

In-vitro Release Studies

100 µg/ml solution of the scaffold was prepared and transferred to a conical flask. To this, a predetermined quantity of buffer medium was added and placed in an orbital shaker. By amassing the buffer fluid from the test tubes, the quantity of chitin expressed out was assessed. The withdrawal aliquot was replaced

at 30 min intervals for 8 h. The amount expelled out was recorded at 390 nm. The discharged amount was ascertained from the standard curve. From this percentage drug release was calculated and percentage drug release was plotted versus time.

In-vitro Antibacterial Activity

Agar disc diffusion method

Preparation of inoculums:

On agar slant, typical cultures were conserved at 4°C. By relocating a coil of cells from the typical cultures to test tubes, lively cultures were developed. The antiseptic action was ascertained by the agar disc diffusion technique.

Antibacterial activity

Predistinct quantity of specialized agar medium was solubilised in a predefined quantity of water. To the above blend, agar was added over again. It was then disinfected, poured into sanitized petri plates and allowed to harden for 1 h, succeeded by dispersal of inoculum. Discs were prepared with test specimen (S5) 20 µl sample of respective concentrations (250 µg, 500 µg, 1000 µg), negative control sterile distilled water and positive control 10 µl (10 µg) streptomycin. Plates were then exposed for hatching. Area of inhibition was then recorded.

RESULTS AND DISCUSSION

In recent years, chitin has been used in various drug delivery systems by several *in-vitro* and *in-vivo* studies. Certain derivatives such as chitosan gel, nanosuspension, hydrogels, nano-fibrous chitin and scaffolds are the emerging and profound way of drug delivery for wound healing. Recently the efficacy of chitosan gel was identified and reported that 5% chitosan increased collagen synthesis and stabilization at the wound site. Similarly, chitin hydrogel/n ZnO bandage was synthesized and evaluated for the antibacterial wound dressing. The study revealed that nano ZnO helped to achieve the antibacterial effect and this bandage can be used in case of burns and diabetics. In 2015, Ribeiro *et al.* evaluated the applicability of chitosan hydrogel as a wound dressing. In this study, the prepared formulations were characterized as follows,

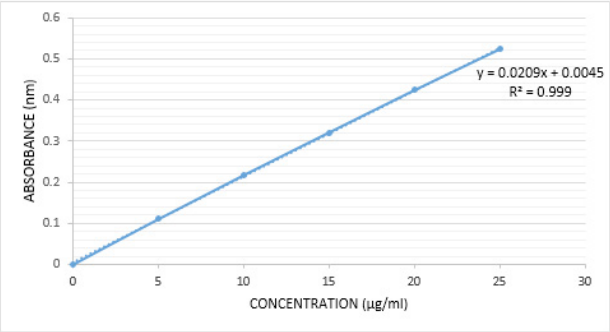


Figure 1: Calibration curve of chitin.

Table 2: Calibration data of Chitin.

Concentration (µg/ml)	Absorbance (nm)
0	0.0000
5	0.1104
10	0.2173
15	0.3207
20	0.4236
25	0.5231

Table 3: Weight loss data of scaffold formulations.

Time (Days)	S1(%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)
1	1.8	1.5	0.2	0.1	0.0
3	3.2	2.7	1.2	0.9	0.0
7	4.0	3.9	2.2	1.7	0.5
15	5.7	4.3	3.1	2.4	1.2
21	6.5	5.5	4.2	3.3	1.9
28	7.0	6.0	5.1	4.0	2.7

Fabrication of Scaffolds

All five formulations of scaffold namely S1, S2, S3, S4 and S5 were formulated according to Table 1 and subjected to various characterization studies.

Calibration Curve of Chitin

As shown in Table 2 and Figure 1, the calibration curve was found to be linear in the concentration range of 5-25 µg/ml.

Weight Loss

From the above results, as shown in Table 3 and Figure 2, Scaffold S1 exhibited a maximum weight loss of 7%. Scaffold S2 exhibited a weight loss of 6% during the period of study. The scaffold S4 showed less weight loss compared to S3. Scaffold S5 showed the least loss of weight (2.7%).

Swelling Ability

As shown in Table 4, the swelling ability was found to be increasing with an increase in chitin concentrations. The swelling ability is important when the scaffold is hired in a large area; the sooner the better. The S5 formulation has a higher swelling ability when compared to other formulations.

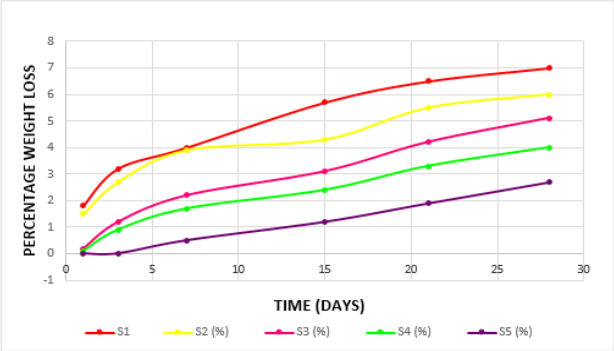


Figure 2: Weight loss graph of scaffold formulations

Table 4: Swelling ability of scaffold formulations.

Formulation Code	W_i (g)	W_f (g)	Swelling Ability (%)
S1	1.00	2.12	112
S2	1.00	3.10	210
S3	1.00	4.60	360
S4	1.00	5.20	420
S5	1.00	6.40	540

Table 5: Porosity measurement data of scaffold formulations.

Formulation Code	W_w (g)	W_d (g)	W_i (g)	Porosity (%)
S1	0.45	0.25	1.10	30.76
S2	0.58	0.25	1.15	57.89
S3	0.59	0.25	1.19	56.66
S4	0.62	0.25	1.20	63.79
S5	0.65	0.25	1.24	67.79

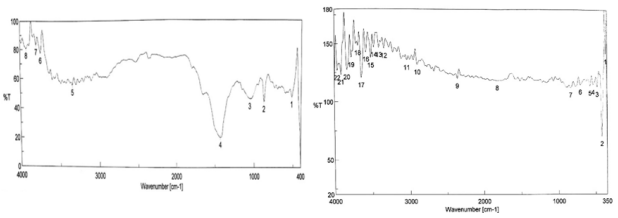


Figure 3: FT-IR spectrum of Chitin and S5 scaffold.

Porosity Measurement

As shown in Table 5, the porosity was high when chitin concentration was high. The porosity was found to be greater for S5 than all the other formulations.

FT-IR Analysis

As shown in Figure 3, the FT-IR spectrum of chitin and S5 scaffold showed no specific drug interaction between the drug and excipients and hence it can be further developed into various formulations.

Optical Microscopy

Figure 4 depicted the S5 scaffold and they were found to be wing-shaped.

Scanning Electron Microscopy

The images of Figure 5 exhibited that the scaffold was found to have a very porous structure with smooth surface morphology.

Transmission Electron Microscopy

The images of TEM as depicted in Figure 6 showed that the scaffolds were porous and spherical.

X-Ray Diffraction

As given in Figure 7, the formulated scaffold was found to exhibit a crystalline structure.

Zeta Potential

As depicted in Figure 8 and 9, the size distribution was found to be 1372 d.nm and zeta potential was found to

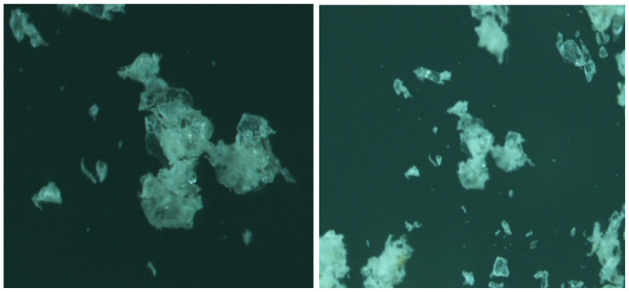


Figure 4: Images of optical microscopy.

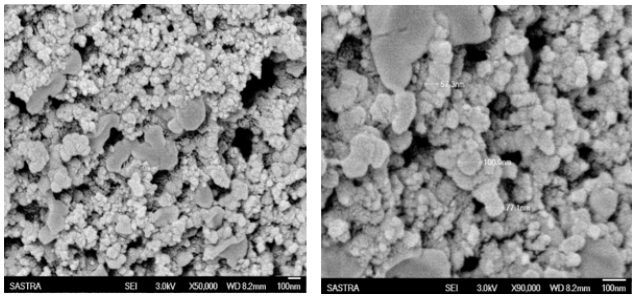


Figure 5: Images of SEM.

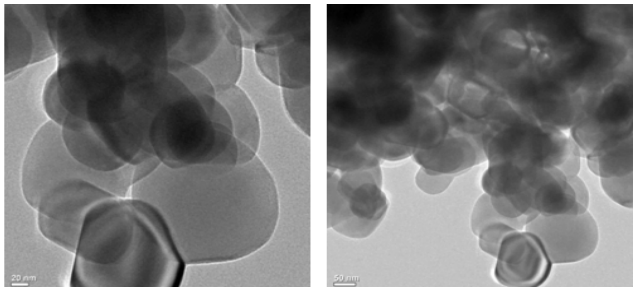


Figure 6: Images of TEM.

be a positive value of 12.2 mV which indicated good stability of the scaffold.

In-vitro Drug Release Studies

As shown in Figure 10, the scaffold S1 showed an initial burst release and the release rate was found to be 100% at the end of 5 h, whereas scaffolds S2 showed 100% release at the end of 5.5 h and S3 showed the release of 98% at the end of 6 h study. The S4 showed the release of 100% at the end of 7 h. However, S5 showed sustained release profile over an extended period of study for up to 8 h. Hence, the formulation of S5 has been optimized for further analytical studies.

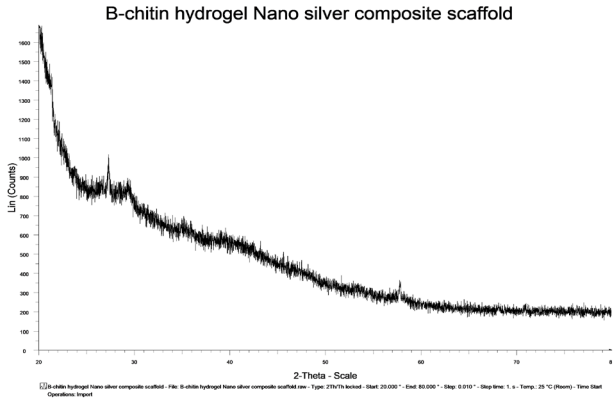


Figure 7: XRD Graph.

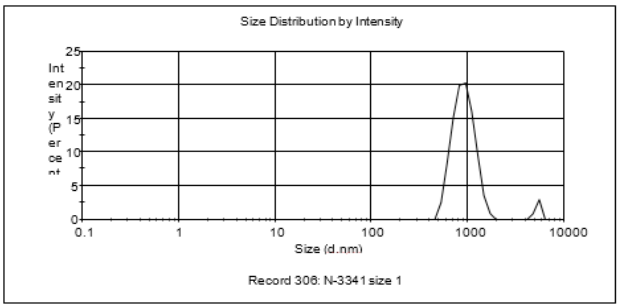


Figure 8: Size distribution graph.

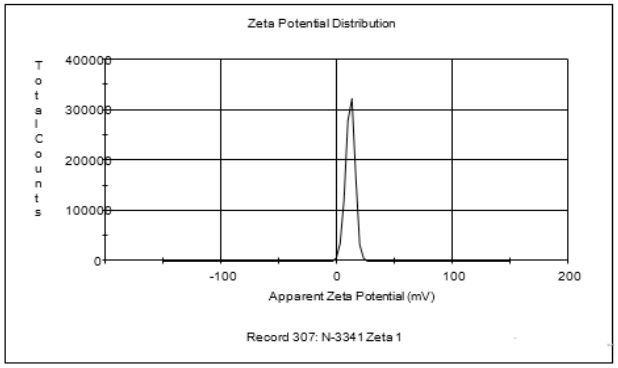


Figure 9: Zeta potential distribution graph.

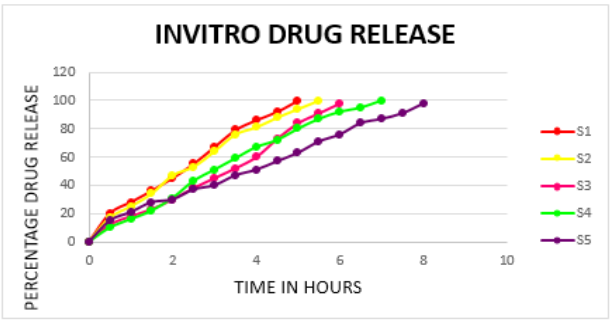
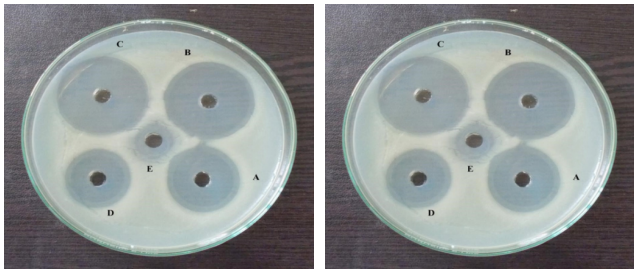


Figure 10: *In-vitro* drug release profile graph.



(a) *S. typhi*
A – 250 µg of scaffold
B – 500 µg of scaffold
C – 1000 µg of scaffold
D – Sterile distilled water (negative control)
E – Streptomycin (positive control)

(b) *S. aureus*
A – 250 µg of scaffold
B – 500 µg of scaffold
C – 1000 µg of scaffold
D – Sterile distilled water (negative control)
E – Streptomycin (positive control)

Figure 11: *In-vitro* antibacterial activity against *S. typhi* and *S. aureus*.

Table 6: *In-vitro* antiseptic action results.

Micro-organisms	Area of Inhibition in mm				
	250 µg	500 µg	1000 µg	Sterile distilled water	Streptomycin 10µg
Chitin scaffold					
S.typhi	26	32	35	23	18
S.aureus	25	30	34	22	16

No. of Microorganism: 4 *(Salmonella typhi and Staphylococcus aureus)*
Sample concentration: 250µg, 500 µg, 1000µg
Negative control: Sterile distilled water
Positive control: Streptomycin (disc.10 µg)

In-vitro Antibacterial Activity

Table 6 and Figure 11 (a, b) showed the results of antiseptic activity studies. Figure 11a showed bactericidal proficiency of chitin nano silver fused scaffold against gram-negative *S. typhi* while Figure 11b showed activity against gram-positive *S. aureus*. It was found that zone of inhibition was higher towards *S. typhi* than *S. aureus* signifying greater proneness of gram-negative microbes to chitin nanosilver fused scaffold. Also as the concentration of nanosilver compound scaffolds was amplified, the area of reticence was also found to be

amplified. These outcomes showed that sterile activity was due to the existence of Nanosilver and chitin. Hence, the developed scaffolds exhibited good antibacterial activity.

CONCLUSION

The scaffold is a versatile bioactive product among wound dressing materials, whose production is flexible and economic. The present work was aimed towards synthesizing chitin from crab shell, followed by preparation of chitin hydrogel and nanosilver solution which was then fabricated into chitin hydrogel nanosilver fused scaffold by varying concentrations of chitin. Chitin holds great action as a wound curative accelerator. The wound curative capability and antiseptic activity of chitin were further boosted by the adding of silver nanoparticles. Five formulations were developed (i.e., S1, S2, S3, S4, S5) using various concentrations of chitin as mentioned earlier. The prepared scaffolds were studied for its characteristic properties such as weight loss, swelling ability, porosity measurement and *in-vitro* release studies. The augmented formulation i.e., S5 was subjected to further characterization studies such as FT-IR analysis, optical microscopy, SEM, TEM, XRD, zeta potential and *in-vitro* antibacterial activity.

Owing to the greater water uptake activity, sufficient porosity, improved antibacterial activity and extended drug release, the chitin hydrogel nanosilver fused scaffold would be a hopeful biomaterial for wound dressing applications. From this research, it was concluded that the chitin hydrogel Nanosilver fused scaffold is a viable alternative to existing conventional dosage forms which lead to improved bioactivity and a promising biomaterial for wound dressing applications in case of administration affords resulting in better patient compliance and cost-effective therapy in the field of biomedical application. Future studies include the study of *in-vitro* cytotoxicity studies and *in-vivo* animal experimental models.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

FT-IR: Fourier Transform Infra-Red Spectroscopy; **SEM:** Scanning Electron Microscopy; **TEM:** Transmission Electron Microscopy; **XRD:** X-Ray Diffraction; **SBF:** Simulated Body Fluid; **µg:** Microgram; **nm:** Nanometer; **ZnO:** Zinc oxide; **mV:** millivolt.

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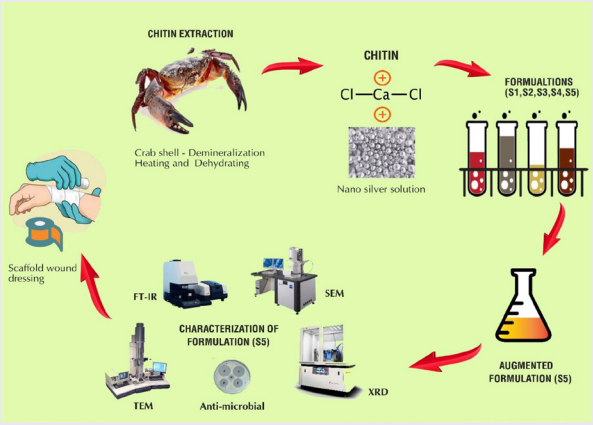
SUMMARY

- The present work was designed to fabricate and characterize the chitin hydrogel nanosilver fused scaffold for wound dressing application. The chitin was obtained as a result of treating crab shell with 7% HCl and heating it upto 300°C. Further clarification was done by dehydrating.
- 1 g, 2 g, 3 g, 4 g and 5 g of chitin were accurately weighed and mixed into five beakers containing each 1 litre of saturated calcium chloride/methanol and mixed for two days with additional water and clarified using Whatman filter paper. Silver nitrate solution was prepared and mixed with the formulations and lyophilized for two days to obtain chitin hydrogel/nanosilver fused scaffold formulations namely S1, S2, S3, S4 and S5.
- All the formulations were subjected to weight loss, porosity measurement, swelling ability and FT-IR analysis. The results were remarkable that the formulations S5 possessed least loss of weight (2.7%), high swelling ability and greater porosity when compared to the other formulation.
- Further the formulation S5 was subjected to surface analysis and it was found that the scaffold was wing shaped, very porous and with smooth surface. The scaffold possesses a crystalline structure with a zeta potential value of 12.2 mV. Also in the antibacterial study it was found that zone of inhibition was higher towards *S. typhi* than *S. aureus* signifying greater proneness of gram-negative microbes to chitin nanosilver fused scaffold.
- From the results it was evident that the chitin hydrogel nanosilver fused scaffold would be promising biomaterial for the wound dressing applications resulting in better patient compliance and cost-effective therapy.

PICTORIAL ABSTRACT



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