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ORIGINAL PAPER

Preparation and characterization of artificial skin using chitosan and gelatin composites for potential biomedical application

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Abstract A bioadhesive wound-dressing material based on the combination of gelatin and chitosan with a proper ratio was developed and successfully applied in biomedical fields. The composite films were prepared with increase in chitosan concentration in a fixed amount of gelatin and were evaluated for mechanical stability (e.g., tensile strength, elongation-at-break), water and buffer uptake capacity, water and buffer aging, molecular structure, morphology, thermal stability, and for biological properties (e.g., antimicrobial activity, cytotoxicity, in vivo wound-healing performance). It is noteworthy that the 10:3 (gelatin:chitosan) composite films showed the best physico-mechanical, thermal, and antimicrobial properties among the other ratios blend films. The improved mechanical and thermal stability of the 10:3 composite film suggested its promising use as carrier for controlled release drug. The composite film was evaluated using a rat model for in vivo tests to ascertain the applicability of the proper ratio of the chitosan and gelatin in the film for best wound-healing activity. Wound sites dresses with gelatin/chitosan composite films showed excellent rapid healing of the wound surface than those dressed with eco-plaster and gauze. Within a day after dressing with 10:3 composite film, the healing efficiency was found to be 80 %.

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Introduction

The preparation of artificial skin by natural materials such as gelatin, pectin, starch, cellulose, alginate, chitin, collagen, polyamino acids, and dextran has been established to enhance the healing process [1–3]. The structures of these natural materials are analogs of protein and growth factor structures in the human body that may be more relevant for stimulating the appropriate physiological responses required for cellular regeneration and tissue reconstructing in wounds [4]. To protect the artificial skin from infections and dehydration, wound has to be protected from its environment. The wound surface should be kept just damp enough to obtain the benefits of accelerated healing, but there should be no accumulation of fluid between the wound and the dressing because of the risk of infection [5]. Bacterial invasion may make the wound unsuitable for skin grafting due to the growth of bacteria population. Wound dressing has a skin top-layer supported with a sponge-like sub-layer that can meet the requirements such as higher gas permeation and protection of wound from infection and dehydration [6].

Chitosan, a naturally derived polysaccharide, has been extensively studied for biomedical applications and pH-sensitive drug delivery [7, 8]. Chitosan mixed with biological components (e.g., collagen, gelatin, polysaccharides, and glycosaminoglycans) composites have already been used in drug delivery or tissue repairing [9, 10]. Among different biological components, gelatin has been proven to be a promising material to be mixed with chitosan [11]. Gelatin is a well-characterized protein fragment obtained by partial degradation of water-insoluble collagen fiber and has been widely used in the biomedical field, because of its merits, including its biological origin, biodegradability, hydrogel properties, and commercial availability at a relatively low cost [12]. The carboxyl groups on its chain backbones are able to form hydrogen bonds with chitosan to form a well-mixed hybrid. As gelatin is soluble in water, it reacts with covalent cross-linkers to form insoluble gels.

Both gelatin and chitosan are biodegradable, biocompatible, bioactive, low antigenic, and mechanically stronger. The incorporation of chitosan with gelatin has an aim to develop a material which would have good mechanical properties, thermally stable in the human body, good swelling property, effective water absorption capacity, and biocompatible without any side effects to the applied natural system. It also involves the development of a highly volatile solution which would form a membrane at a very short period of time and thus will provide a biocompatible barrier to prevent blood loss followed by injury. The present research work concentrated on the preparation and evaluation of composite films made with chitosan–gelatin blends at different proportions to ascertain the applicability of prepared combination for developing a biomedical material that can be used as wound-dressing material.

Experimental

Film preparation

Pure chitosan solution

Chitosan (M.W. 160.9) was purchased from MERCK, India. The different compositions (1, 3, 5, and 7 %) of chitosan were prepared by dissolving required amount of chitosan in 2 % acetic acid with constant shaking at 50 °C for 30 min.

Pure gelatin solution

Pure gelatin (M.W. 10,000 g/mol) was obtained from MERCK, India. The fixed amount of gelatin (10 %) was prepared by dissolving 10 g gelatin in 90 g water. The resulted solution was placed in a hot plate with constant stirring by a magnetic stirrer wherein the solution was heated at 80 °C for 40–60 min.

Composite preparation

Different composites were prepared by blending different compositions (1, 3, 5, and 7 %) of chitosan with 10 % gelatin in a hot plate with constant stirring by magnetic stirrer for 30 min. The obtained solution was placed in a thin plate for casting. After 24 h, different composite films (gelatin:chitosan) of 10:1, 10:3, 10:5, and 10:7 were obtained.

Buffer solution preparation

8 g sodium chloride (NaCl), 0.2 g potassium chloride (KCl), 1.44 g sodium biphosphate (Na_2HPO_4), and 0.24 g potassium biphosphate (K_2HPO_4) were added in 1,000 mL deionized water to prepare the buffer solution.

Characterizations

Water uptake and water aging

Pure chitosan, pure gelatin, and all composites were soaked in water for different times at room temperature (30 °C). Then all the films were dried at 30 °C for determination of water aging.

$$\text{Water uptake } (\%) = (W_a - W_b)/W_b \times 100 \quad (1)$$

$$\text{Water aging } (\%) = (W_a - W_d)/W_a \times 100 \quad (2)$$

where W_a and W_b are the weights of the sample after and before soaking, respectively; W_d is the weight of the dried sample.

Buffer uptake and buffer aging

Pure chitosan, pure gelatin, and all composites were soaked in buffer solution for different times at room temperature (30 °C). Then all the films were dried at 30 °C for determination of buffer aging.

$$\text{Buffer uptake } (\%) = (W_a - W_b)/W_b \times 100 \quad (3)$$

$$\text{Buffer aging } (\%) = (W_a - W_d)/W_a \times 100 \quad (4)$$

where W_a and W_b are the weights of the sample after and before soaking, respectively; W_d is the weight of the dried sample.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectroscopy of gelatin/chitosan blend films was performed by ATR-FTIR spectrophotometer (Model RX1, PerkinElmer, UK) in the wavenumber range 400–4,000 cm^{-1} with resolution of 4 cm^{-1}. The FTIR spectrum was taken in a transmittance mode.

Morphological study

The morphological study of the gelatin/chitosan blend film was done by scanning electron microscope (model XL 30, Philips, The Netherlands). In this machine, the platinum-coated composites samples were kept in an aluminum disk plate. A computer was integrated with the machine with relevant software. Scanning electron micrographs of the sample was obtained from computer.

Thermal analysis

Thermal analysis included a group of techniques wherein some physical properties of the sample were monitored under controlled conditions with variation of temperature at a programmed rate. The mass change of the film was monitored using thermogravimetric analysis (TGA). The heat absorption was monitored using differential thermal analysis (DTA). Together, they represent a powerful method of analysis, differential thermogravimetry (DTG). The thermal test of the films was taken using computer-controlled TG/DTA 6300 system controlled to an EXSTAR 6000 STATION, Seiko Instrument Inc., Japan. The TG/DTA module used a horizontal system balance mechanism. All the experiments were performed under nitrogen atmosphere.

Differential scanning calorimetry (DSC) is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and a reference is measured as a function of temperature. DSC tests were carried out using a DSC-60 (SHIMADZU Corp, Japan). The sealed aluminum pan was put on the calorimeter along with an empty sealed pan. Change of heat per gram of sample was recorded at a constant temperature for 60 min with a computerized

system in dry nitrogen environment. Three different isothermal DSCs were taken at 303, 318, and 333 K temperatures for each sample.

Tensile properties testing

Tensile strength (TS) and percent elongation-at-break (EB) of the cured films were measured with Universal Testing Machine (Hounsfield, model H50 Ks 0404, UK). The efficiency of the machine was within ±1 %. The cross-head speed was 10 mm/min and the gauze length was 20 mm with load capacity of 500 N. Five different blends with different concentrations of chitosan in constant amount of gelatin were analyzed using universal testing machine. The physico-mechanical properties of the composite films (gelatin:chitosan) of 10:1, 10:3, 10:5, and 10:7 at 65 % relative humidity and at room temperature (30 °C) were investigated.

$$\text{Tensile strength TS (MPa)} = \frac{\text{Load (N)}}{\text{Thickness (mm)} \times \text{Width (mm)}} \tag{5}$$

$$\text{Elongation-at-break, EB (\%)} = \frac{\text{Displacement at break} \times 100}{\text{Gauze Length}} \tag{6}$$

Microbial sensitivity analysis

Nutrient agar for bacterial culture and potato dextrose agar for fungal culture were prepared. One loop culture from the stock cultures of *Bacillus* sp. and *Candida* sp. were inoculated in normal saline. Then these cultures were subjected for inoculation onto their corresponding media using cotton bud. After inoculation, the gelatin film, the chitosan film, and the composite films of 10:1, 10:3, 10:5, and 10:7 were placed onto bacterial and fungal smear. Then the plates containing pure gelatin, pure chitosan, and composites of various concentration were incubated at 37 °C for 24 h to observe their inhibition effects against the bacteria and at 30 °C for 48 h to observe their inhibition effects against fungus.

In vitro cytotoxicity study

In vitro cytotoxicity test was performed using brine shrimp lethality bioassay method. It is a primary toxicity screening procedure used as an initial screening of bioactive compounds. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial seawater and pH was adjusted to 8.5 using 0.1 N NaOH under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. The best composite film (10:3) was dissolved in artificial seawater at 0.25, 0.50, 1.00, 2.00, 4.00 mg/mL concentration and was taken in Petri plates where the active nauplii were inoculated. After overnight incubation, the nauplii were counted. 0.5 mg/mL of vincristine sulfate (an anticancer drug) was considered as positive control.

Wound-healing studies using rat model

In rat model, artificial wound was formed surgically and dressed with a gelatin/chitosan film plus eco-plaster and gauze. Wound healing was assessed by monitoring wound contraction, re-epithelialization, and wound morphology (real time photograph). Wound-healing efficiency was measured using the following formula:

$$\text{Healing Efficiency} = (\text{Total Healed Area}/\text{Total Wound Area}) \times 100 \quad (7)$$

Results and discussion

Physico-mechanical analysis

TS and EB

Cross-linked gelatin gels are used as biomaterials in living tissues, either as bioadhesives or as devices for sustained drug release. As these applications involve surgical insertion of gels, the effect of cross-linking on mechanical properties is relevant. The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters: TS and EB. It is required that composite films must be flexible and strong to be used as potential wound-dressing materials. Figure 1 shows the TS and EB of the composite film in both wet and dry conditions as a function of the percentage of chitosan. Note that the values of TS and EB for gelatin film in wet state were considered to be zero because its mechanical properties were too weak to measure. The TS and EB of the film increased initially with the increase of chitosan content suggesting that the mechanical properties of gelatin were improved by blending with chitosan. In both wet and dry states, composite film exhibits a lower modulus and a higher percentage of EB compared to gelatin film. The maximum TS occurred at about 10:3 ratio which correspond to the optimum interactive ratio between chitosan and gelatin and the additional chitosan caused the decrease of TS and EB. The soft and elastic complex with 10:3 ratio of gelatin and chitosan have a lower elasticity and a higher percentage of EB compared with gelatin suggesting that the composite of 10:3 ratio is suitable biomaterial for regeneration of soft nerve tissue, in terms of coordination of their mechanical properties.

Water and buffer uptake

Water and buffer uptake ability is one of the basic requirements of an artificial skin. It is important for the absorption of body fluid and for a transfer of cell nutrients and metabolites through the materials [13]. Water and buffer uptake studies of different composites comprised of gelatin and chitosan (Fig. 2) showed that the water and buffer uptake capacity of the composite films were decreased with increasing amount of chitosan. Pure chitosan is not soluble in water. In contrast, pure gelatin is

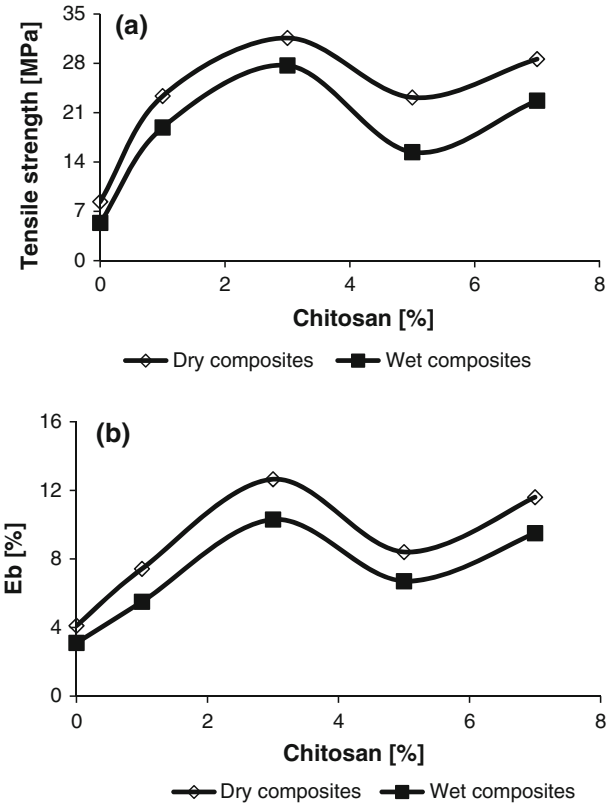

Fig. 1 Variation of **a** tensile strength and **b** EB of composite film with the percentage of chitosan. The percentage of gelatin in all of the studied composite films was kept constant (10 %)

a hydrophilic polymer with very high water uptake (200 %) and it lasted just for 10 min before dissolving completely. When chitosan mixes with gelatin, the hydrophilicity of the composite films is decreased (i.e., the stability of the films is increased). In this study, the 10:1, 10:3, and 10:5 composite films can retain larger amounts of water for longer holding time (40 min), making the film softer. The tight aggregation of chitosan chains in the composite film might make the materials stable in sizes and shapes [13]. By further addition of chitosan (e.g., 7 %) to the composite film, the hydrophilicity of the film was significantly decreased (See Fig. 2) that led to a decrease in the film porosity. It is indicated that there was no significant improvement in the stability of the composite film by further addition of chitosan (e.g., 7 % or more).

Buffer uptake is lower for chitosan and very high (160 %) for gelatin. The composite film with 10:3 and 10:1 (gelatin:chitosan) ratios showed maximum buffer uptake (140 %) with greater shrinkage attributed to their comparatively higher material contents that aided in swelling and retention of fluids. Because of the less porosity, a decreased buffer uptake of the composite film was observed when the ratio of gelatin:chitosan is 10:7.

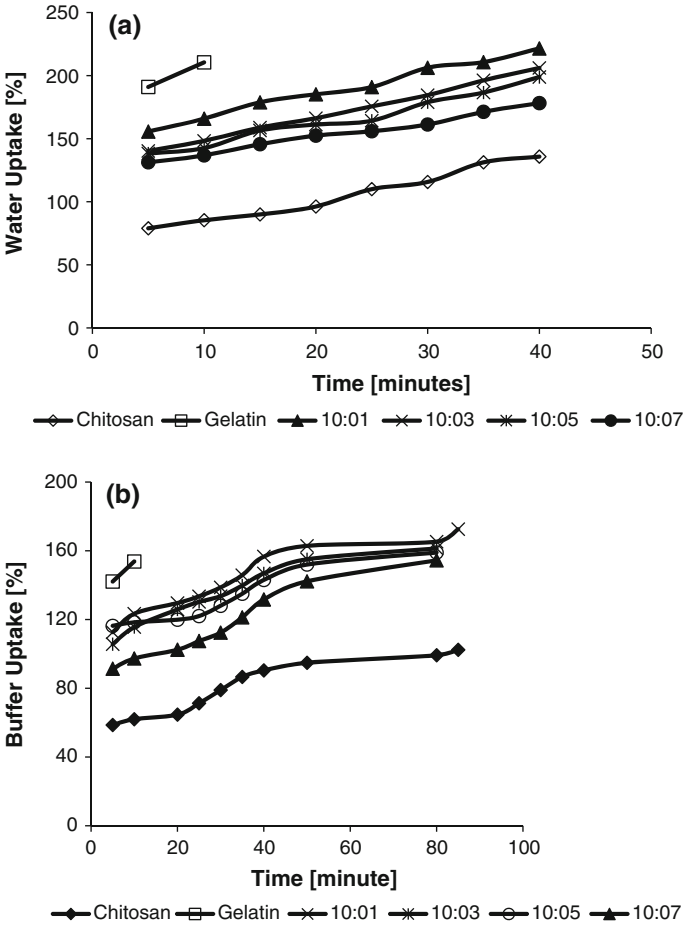

Fig. 2 **a** Water uptake, **b** buffer uptake of pure chitosan, pure gelatin, and their composite films with respect to time

Water and buffer aging

Water and buffer aging studies (Fig. 3) showed that composite films containing 1 % chitosan had higher percentages of water and buffer aging. In contrast, films containing 3 % and even more percentage of chitosan had lower percentages of water and buffer aging. So, the composite films (gelatin:chitosan) of 10:3, 10:5, and 10:7 showed lower water and buffer aging indicating better wound-dressing films.

FTIR study

Figure 4 shows the FTIR spectra of chitosan, gelatin, and the chitosan–gelatin composites. The spectrum of chitosan showed peak of O–H at 3,200–3,500 cm^{-1}, peaks around 905 and 1,153 cm^{-1} of assigned saccharine structure and a strong

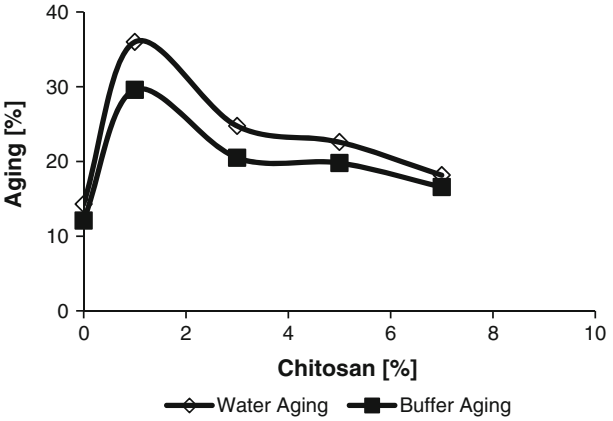

Fig. 3 Water and buffer aging of composite films with the percentage of chitosan. The amount of gelatin in all of the studied composite films was kept constant (10 %)

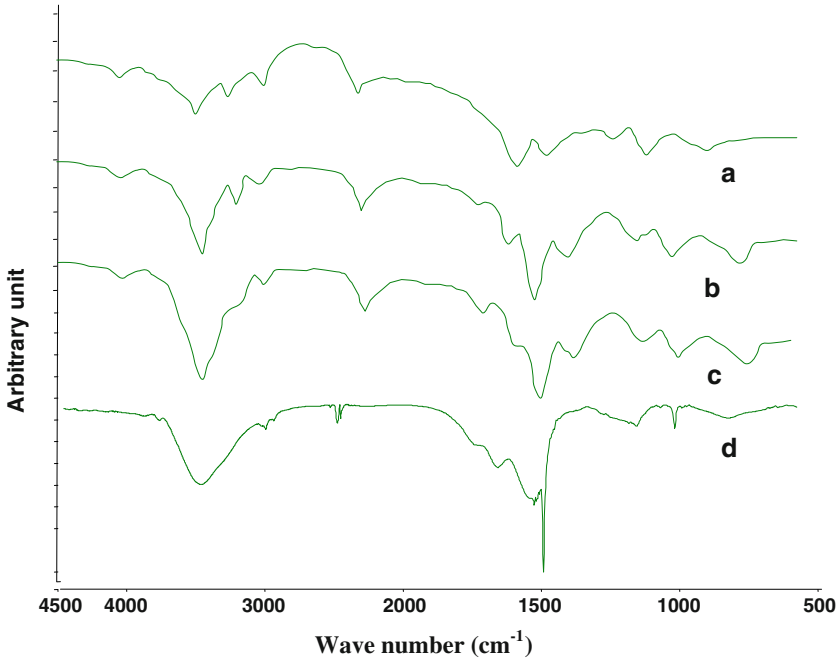

Fig. 4 FTIR spectra of (*a*) pure gelatin film, (*b*) composite 10:3, (*c*) composite 10:5, (*d*) pure chitosan

amide characteristic peak at 1,650 cm^{-1} as well as an amino characteristic peak at around 1,570 cm^{-1}. The spectrum of pure gelatin showed the peak in the region of 3,000–3,600 cm^{-1} for O–H group, at 1,580 cm^{-1} for amino band, and at 1,656 cm^{-1} for the carbonyl peak of the amide group. The band identified as

C–H is at 2,800–3,000 cm^{-1}; also N–H stretching vibrations may be due to overlapping with O–H bond in the range of 3,100–3,500 cm^{-1}.

The shift of hydroxyl, carbonyl, and amino bands in the spectra of chitosan–gelatin composite showed the evidence that chitosan and gelatin formed a hydrogen bonding involved complex. The blend showed double peaks at 1,680 and 1,630 cm^{-1}, and another peak at 1,538 cm^{-1}. This might be due to some degree of amidization between the ammonium ($–NH_3^+$) ions of the chitosan and the carboxylate (COO^-) ions of the gelatin by the partial conversion of electrostatic bonds into chemical bonds (condensation reaction) as discussed by Bernable et al. [14]. However, the bands are much broader in the gelatin/chitosan-blended films (10:3 and 10:5) than the film of gelatin itself. This explains the increase of crystallinity of higher amount of chitosan in the film. Composite 10:3 and 10:5 films led to small modifications in the spectrum of gelatin due to the shifting of carbonyl, O–H and N–H bands. Also in the two composites, decreased intensity at those regions indicating cross-linking between gelatin and chitosan has been occurred to form composite film.

Morphological study

The correlation of the phase and chemical structure with the morphology is important to understand the properties of gelatin/chitosan composites as an artificial skin. The morphologies of pure chitosan, pure gelatin, and their composite films were studied with scanning electron micrograph (SEM).

The surface of pure gelatin membrane was found rough and uneven (Fig. 5a). In contrast, the surface of pure chitosan membrane was less rough and less uneven (Fig. 5b). The micrograph shows that the chitosan has crystalline flat lamellae with leaf-like shape. Large spaces between the leaf-like structures are available throughout the surface membrane. The crystalline structure was able to form due to longer evaporation time taken which enables it to crystallize. However, for the membrane with gelatin/chitosan blend ratio of 10:3, a much plainer texture was observed (Fig. 5d). It is attributed that chitosan could ameliorate the smoothness of the surface of gelatin film. The micrograph shown had proven that inclusion of 10:3 ratio of gelatin and chitosan in the blend does contribute to the increment of plainness at the membrane surface. The surface of composite 10:1 membrane showed a poorer plainness (Fig. 5c) compared with composite 10:3 membrane. Thus, the addition of chitosan at the right ratio has improved the morphology of gelatin.

Thermal analysis

Thermogravimetric analysis (TGA)

TGA is a continuous process, involving the measurement of sample weight in accordance with increasing temperature in the form of programmed heating. As TGA provides better understanding of thermal decomposition behavior, the thermal stability and thermal decomposition of gelatin, chitosan, and composite films were

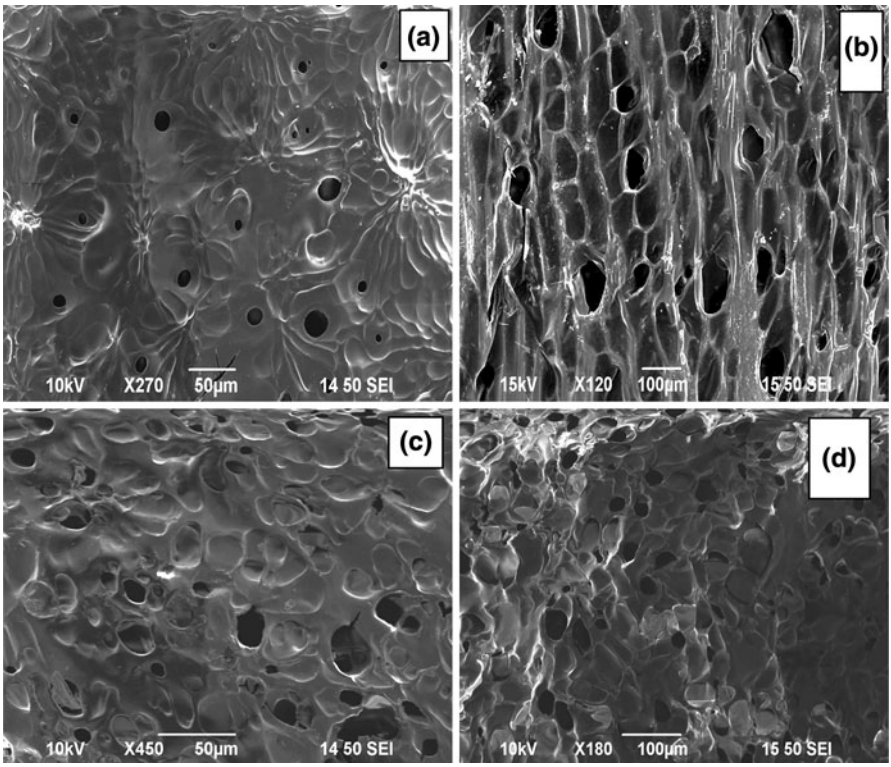

Fig. 5 SEM of **a** pure gelatin, **b** pure chitosan, **c** composite 10:1, **d** composite 10:3

investigated using TGA and the results are presented in Fig. 6. Pure chitosan showed one step of weight loss located at 258.37 °C (Fig. 6a). The curve of pure gelatin showed two zones of weight loss. The first weight loss at 283.6 °C was due to the loss of water; the second weight loss was at 524.64 °C (Fig. 6b), showing that thermal degradation of gelatin took place. The composite film (10:1) showed its thermal weight loss at 269.43 °C due to the moisture loss and at 451.15 °C keeping undegraded films of 41.20 % (maximum weight loss) which are believed due to the disintegration of intermolecular and partial breaking of the molecular structure (Fig. 6c). Similarly, the composite films (10:3) and (10:5) showed their thermal weight losses at 278.63 and 265.79 °C, respectively, and the second weight loss at 460.67 and 470.27 °C keeping undegraded films of 42.72 and 43.63 %, respectively (Fig. 6d, e). All these results indicate that the thermal stability of the composite has been improved by incorporating chitosan to the gelatin.

Differential scanning calorimetry (DSC)

Figure 7 shows the DSC thermograms of gelatin, chitosan, and blended samples with chitosan contents ranging from 1 to 5 %. For pure gelatin, four endothermic phase transitions were observed at about 100.3, 154.18, 260.47, and 327.36 °C and

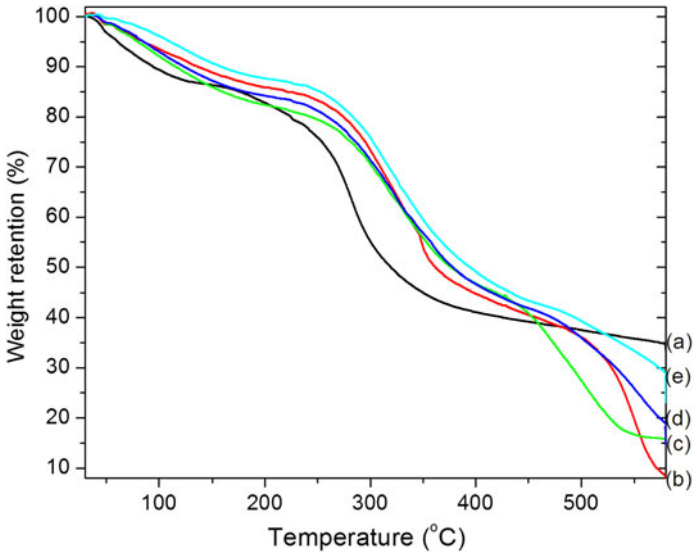

Fig. 6 Comparison of TGA graphs of (*a*) pure chitosan, (*b*) pure gelatin, (*c*) composite 10:1, (*d*) composite 10:3, (*e*) composite 10:5

these were associated with enthalpies of 15.27, 9.95, 1.36, and 0.27 J/g, respectively. The melting point of gelatin was found to be around 320 °C. Therefore, it was suggested that the endotherm observed at 327.36 °C (Fig. 7a) due to the melting phase transition of gelatin. The endothermic peak at 100.3 °C was probably associated with the denaturation of the gelatin segments. The endothermic phase transition peak at 100.3 °C may have been related to the helix–coil transition, which was overlapped with the glass transition temperature. The DSC thermogram for pure chitosan (Fig. 7b) showed one significant endothermic peak at about 93.93 °C and another significant exothermic peak at about 297.02 °C, which may have corresponded to T_g and the melting transition temperature, respectively. The T_g peak was broad and the melting peak was a sharp one. The broad glass transition was apparently due to the non-equilibrium structures of this process. It is of particular interest to estimate how the thermal transitions of gelatin varied with the chitosan content in the blends. One key aspect was the determination of whether the resulting blended samples were compatible, that is, if they were homogeneously mixed at the molecular level. Gelatin and chitosan possessed T_g's that were quite close to each other, and this could cause overlapping of their individual transition peaks. The DSC thermograms of all of the blended samples (Fig. 7c–e) showed one single broad glass transition peak with the maximum temperature within the range 87–101 °C. However, the clearly single T_g may have been due to the difficult resolution of overlapped T_g transition for the two blended samples. However, the positions of T_g in the blended systems were found to be an intermediate of the two pure polymers, including the compatibility of the blended systems. In addition, the transition width of the T_g for the blends was almost identical to those of the pure components, which further supported single-phase behavior in the blends.

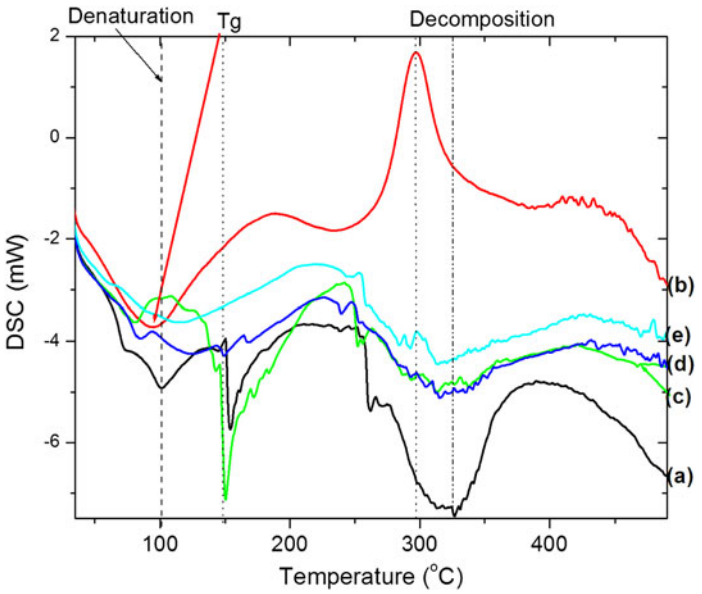

Fig. 7 DSC analysis of (*a*) gelatin, (*b*) chitosan, (*c*) their composite 10:1, (*d*) composite 10:3, (*e*) composite 10:5

Antimicrobial sensitivity analysis

The composite designated as 10:1, 10:3, and 10:5 has antibacterial effect as clear zone of inhibition was found underneath and in the vicinity of these films placed onto the bacterial culture (Fig. 8). It was also found that, there was no antifungal effect of these films shown in the figures. These findings predicted that the wound dressing with the composites could achieve a sustainable antimicrobial effect against both *Bacillus* sp. and *Candida* sp. which are known to infect damaged skin.

Cytotoxicity

In vitro cytotoxicity study on the composite film of 10:3 gelatin/chitosan showed that the possibility of death of nauplii due to toxicity was very low as the number of death was nil for lower concentrations (Table 1). Moreover, gelatin and chitosan were used as parent materials of the scaffold which were both biocompatible. So, the most possible reason for the death of nauplii is the formation of the viscous layer on the gills of the nauplii as the highly concentrated solution of the gelatin-based scaffold led to high viscosity and thus tends to formation of gel-like structure on the gills which eventually inhibit oxygen permeability of the gills and caused death.

In vivo wound-healing studies using the rat model

The composite films performances as wound-healing materials have been observed using 5-day rat model experiment and the results have been shown in Fig. 9. During

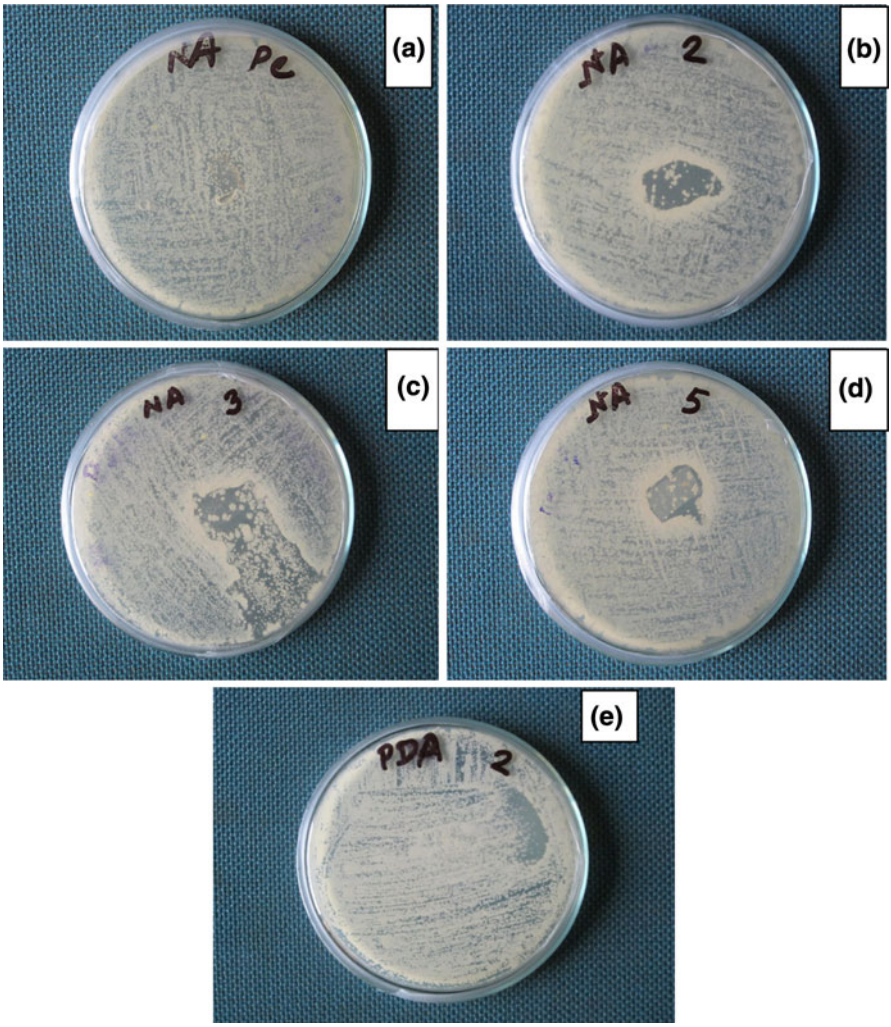

Fig. 8 Antimicrobial sensitivity of **a** pure chitosan, **b** composite 10:1, **c** composite 10:3, **d** composite 10:5, **e** pure gelatin

wound healing with composite films, no significant weight loss or fever was found. The edges of the wound pull inwards to reduce the overall wound area. Wound contraction [15] and re-epithelialization [16] were already established as measures for monitoring wound closure and healing. In this study, wounds supplemented with composite materials, using ecoplast as the standard occlusive dressing, had improved wound-healing results compared to those wounds with the ecoplast alone. In addition, the underlying fibro-vascular tissue appeared more rapidly than for those wounds treated with ecoplast alone. From this experiment, healing

Table 1 Mortality of Brine shrimp (*Artemia salina*) nauplii at different concentration of gelatin/chitosan thin film

Sample no.	Sample name	Dose (mg/mL)	No. of nauplii present after incubation	Mortality (%)
01	Positive control (Vincristine sulfate)	0.5	0	100
02	Negative control (artificial sea water)	–	10	0
03	10:3 Gelatin/chitosan film	0.25	10	0
04	10:3 Gelatin/chitosan film	0.50	10	0
05	10:3 Gelatin/chitosan film	1.00	8	15
06	10:3 Gelatin/chitosan film	2.00	7	28
07	10:3 Gelatin/chitosan film	4.00	5	37

efficiency and the control healing status was found to be 80 and ~5 % after 1 day. On the fifth day, the composite dressing were removed from the wound surface without causing further trauma and the wound surface was found almost totally healed up (Fig. 9f). But for the control dressing, fibrin and blood penetrated the large interstices of the fabric, hardened, and stuck firmly to the wound bed that leads to considerable tissue trauma, and bleeding during removal. As a result, a delayed healing (e.g., the healing efficiency was only 40 % after 5 days) was observed for control dressing. For composites, there was no bacteria creation in the wound region and its surroundings.

Conclusion

The results reported in this study showed that chitosan could be usefully added to gelatin for preparation of soft and elastic complex that has good characteristics of efficient tissue scaffold. FTIR analysis showed that intermolecular interactions existed between gelatin and chitosan, and these interactions led to the good compatibility among the composites. The thermomechanical properties have been drastically improved due to chitosan content in composite films. The tensile test showed that the 10:3 composite films had the highest tensile strength and the highest percentage of EB. DSC analysis showed that the glass transition point increased almost linearly due to increasing chitosan content in gelatin film. The TGA study also showed that the 10:3 composite film was thermally more stable than the other composite films. The development of micro-pores in the blend membrane and the development of smooth and compact surfaces were clearly observed from SEM study. The antimicrobial sensitivity study showed that the composite films have the antibacterial effect though pure gelatin has no antibacterial effect. The gelatin/chitosan composite films showed excellent rapid healing of the wound surface in where the 10:3 composite showed best healing efficiency among the other blend films. This study revealed a successful development and application of a new

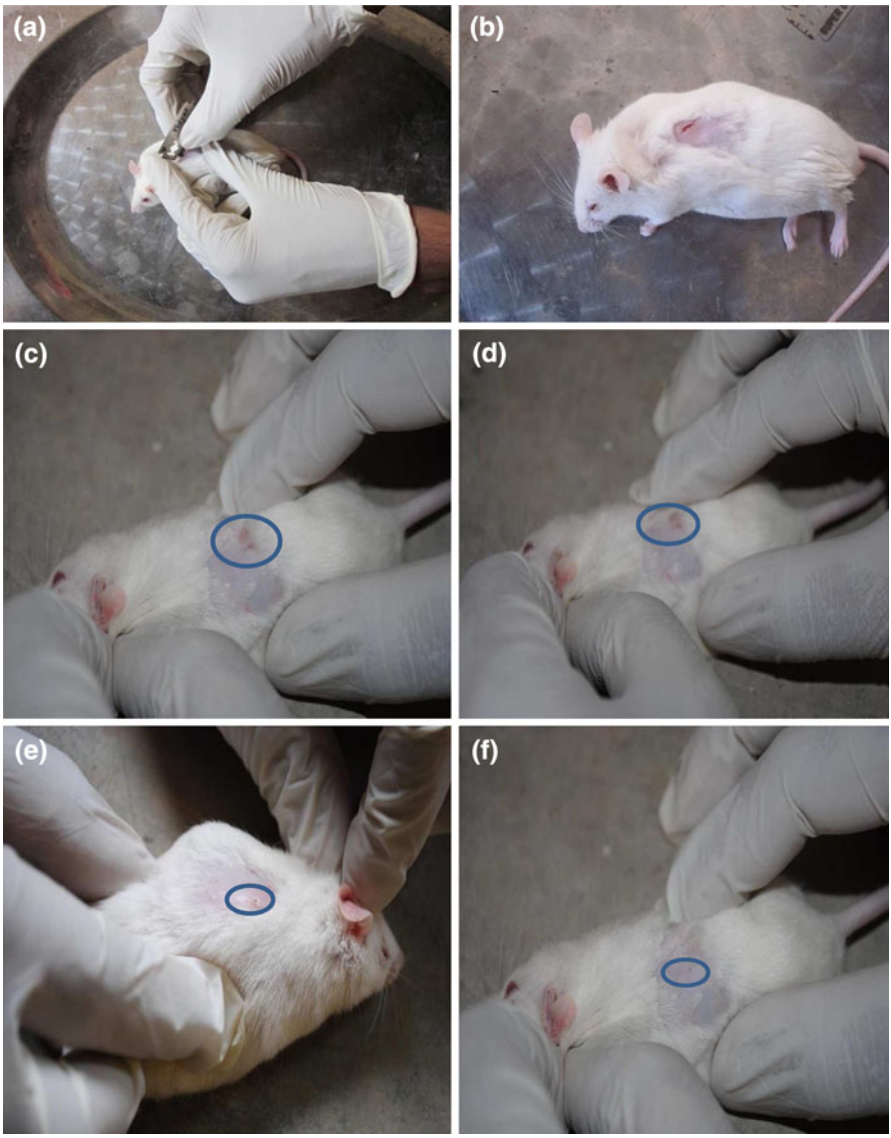

Fig. 9 Rat model experiment: **a** before dissecting, **b** after dissecting, **c** normal bandage (control) after 1 day, **d** normal bandage (control) after 5 days, **e** composite 10:3 after 1 day, **f** composite 10:3 after 5 days. Wound site has been marked with a *circle* in figures (**c**–**f**)

bioadhesive wound-dressing material based on modified gelatin which is more effective that the conventional wound-dressing materials.

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