RESEARCH ARTICLE

Preparation and Biomedical properties of transparent chitosan/ gelatin/honey/aloe vera nanocomposite

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ABSTRACT

Objective(s): Biodegradable polymers are featured with notable potentials for biotechnology and bioengineering purposes. Still, there are limitations in their applicability so that in many cases composite forms are used. The present study is focused on chitosan (CS), gelatin (GEL), honey (H) and aloe vera (AV) for preparation of thin films.

Methods: To prepare the thin film, CS and GEL with ratio of 8 % was used. Similarly, AV, honey and wheat germ oil (WGO) was prepared with 20:20:2 ratio. Afterward, the solution was poured in petri dishes. The dishes were stored at room temperature for 24h until the film was formed. Then, CS/PEO/H, CS/PEO/AV, CS/PEO/H/AV nanofibers electrospinning was done on CS/GEL/H, CS/GEL/AV, CS/GEL/H/AV thin films, respectively.

Results: The results of antibacterial activity, fibroblast cells culture and hemolytic activity were examined. The results of antibacterial tests revealed that thin films containing honey had antibacterial activity against *staphylococcus aureus* and *pseudomonas aeruginosa*. The results of fibroblast cells on the prepared samples indicated more than 90% of the cells are alive. Also, hemolytic activity results indicate that the samples are non-hemolytic.

Conclusions: Considering the biological properties of the thin films, they could be used as a biomaterial for antibacterial wound dressing.

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INTRODUCTION

Wound healing is an autonomous process that might be interrupted in chronic wounds or burn injuries. Biodegradable polymers have many uses in biomedical and bioengineering purposes, such as wound dressing and cancer therapy[1]. However, there are still limitations in their applicability so that in many cases composite forms are used. Thus, finding a dressing that provides coverage and accelerates healing process while preventing infection is of top priorities [2]. The most important features that a suitable dressing must have are preservation of adequate humidity at the would surface, transfer of active combinations, prevention of formation of microbial biofilms, easing pain and inflation, debride dead and damages tissues, and facilitation of reaction between the cells and proteins that can be effective on wound adjustment and healing [3,4]. Since no known material can possess all these specifications, a combination of biopolymers are used to improve wound healing process. Sasikala et al. proposed CS hydrogel membrane and honey as wound dressing [5]. Wang et al. prepared hydrogel of GEL, honey and CS with 20:20:0.5 ratio and obtained the best results in antibacterial and toxicity tests. In addition, the mixture had a significant effect on contraction of wound and acceleration of healing process [6]. Isfandiary et al. used CS /collagen/ AV, as a suitable coverage combination for burns with high cells proliferation performance [7]. Illani et al. used CS and AV gel to prepare nanofiber [8].

CS with low and average molecular weights was used for producing fine film and nanofiber

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respectively. CS is a biocompatible, biodegradable and natural polymer with wound healing effects [9]. One of the most suitable biopolymers for preparing these antibacterial coatings is CS. One of the unique specification of CS is its ability to accelerate formation of granulated tissue along with angiogenesis and orderly sedimentation of fine collagen tissue that leads to complete wound healing. The biochemical effects of CS are activating fibroblasts of cytokine generator and triggering synthesis of collagen type V [10].Also, CS/GEL film is the base for CS / poly(ethylene oxide) (PEO) nanofiber and improves many of its characteristics. Due to Arg-Gly-Asp (RGD) sequence, biological functions of CS, e.g. adhesiveness, cellular migration are improved when it is combined with gelatin. In addition, hydrophilic feature of GEL ensures that the wound is kept moisturized and nutrient and oxygen are available to the wound [11].

On the other hand, herbal solutions like thyme honey and AV have been used to accelerate the wound healing. Several studies have been carried out on antibacterial activity of honey and the antibacterial activity have been confirmed. Thereby, honey is known as a factor for wound healing and antibacterial effects because of features like high osmolarity, low pH (3.5-5), and capability to generate peroxide hydrogen [12-14]. Due to the wide range of chemical compounds (e.g. amino acids, antrokinons (aloin), chromon (aloesine), enzymes, hormons, steroids, minerals, vitamins) AV demonstrates several pharmaceutical properties [15,16]. One of the main effects of AV gel is to keep the wound moisturized and facilitate migration of epithelial cells due to high water content. Polysaccharides of AV improve migration of fibroblast cells, generation of hyaluronic acid and hydroxy proline, and the level of dermatan sulfate in the fibroblasts that play a key role in rebuilding external matrix structure of cell [15, 17, 18].

The purpose of this work is, to provide an antibacterial coating and subsequently to prevent the formation of biofilms. Therefore, the present study is focused on CS, GEL, H and AV for preparation of thin films.

EXPERIMENTAL

Materials

CS with low and medium molecular weight and degree of deacetylation 75-80%, GEL, PEO with a molecular weight of 900.000 $g.mol^{-1}$, glass transition temperature (Tg) of 57°C and melting point of 63-68°C, viscosity: 400–800 CP were purchased from Sigma Aldrich (USA). Glacial acetic acid purity 96% and molecular weight 60.05 $g.mol^{-1}$, twice distilled and deionized water, tween 80 and nutrient agar were purchased from Merck (Darmstadt, Germany). Thyme Honey, AV gel and WGO were purchased from Misivan, Parsiteb and Talayetabiat companies (Iran), respectively. *Staphylococcus aureus* (*S. aureus*) strain (ATCC 29213) and *Pseudomonas aeruginosa* (*P. aeruginosa*) strain (ATCC 29213).

Preparation of thin films

To prepare the thin film, chitosan with low molecular weight and concentration of 1% (w/v) and gelatin with ratio of 8 % was used. The solution container was mounted on a heater-stirrer and 1ml of acetic acid was added while chitosan was being solved. Similarly, AV, honey and WGO was prepared with 20:20:2 ratio. Then, tween 80 (0.25 ml) was added as emulsifier to the solution. The beaker was sealed by parafilm (to avoid evaporation and improve density of the solution). The obtained solution was stored for 5-4h until the polymers were completely solved into the solution. To remove air bubbles, the solution was centrifuged for 20 min (4000rcf). Afterward, 0.2ml ethylene glycol was added as plasticizer and 7ml of the solution was poured in petri dishes (7mm). The dishes were stored at room temperature for 24h until the film was formed along with evaporation of the solution.

ATR-FTIR Spectroscopy

Functional groups and interactions between components of thin film were examined by ATR-FTIR (Perkin Elmer, Spectrum GX).

Evaluation of Antimicrobial Activity

Antibacterial activity was studied using agar plate method, gram-negative bacterium pseudomonas aeruginosa, and gram-positive bacterium *S. aureus*.

At first, nutrient agar microbial culture was prepared following the protocol proposed by the supplier. Two separate suspensions of gram-negative bacterium *P. aeruginosa* and gram-positive bacterium *S. aureus* (1.5×10^8 cfu/ml) were prepared. After preparing the culture and using cotton swabs sterilized by the suspensions, the uniform culture was developed on the surface of plates containing nutrient agar culture.

To survey antibacterial activity of the prepare

films, the samples were cut into 1×1cm pieces and sterilized using UV radiation of biological hood. Then the samples were placed on the plate surface and the plates were kept in incubator (37°C) for 24h. Afterward, diameter of growth inhibition halo around the samples was measured.

Preparation polymeric solution for electrospinning
Preparation polymeric solutions

CS solution was prepared using acid acetic solvent 50% (w/v) in water. At first, 0.2g chitosan powder with average molecular weight was stirred into the solvent. The solution was kept at ambient temperature for 12h until chitosan was solved completely. Then PEO (0.3g) was solved in acid acetic/water solution (10ml) to obtain PEO 3% (w/v).

After preparing CS solution 2% (w/v) and PEO 3% (w/v) in acid acetic/water 50% (w/v) in separate containers, they were mixed at 30:70 ratio and then tween 80 (0.25%) was used as emulsifier. Afterward, honey and AV with ratio of 1% (w/v) was added as additive and the beaker was sealed using para-film (to prevent evaporation and increase density of the solution). Afterward, the solutions were placed in a heater-stirrer with high rpm at 37°C for 24h until all polymer content was solved.

Electrospinning process

For preparing nanofiber were used electrospinning device (Nanoaxma). Each polymeric solution was poured in plastic syringes (5ml) with internal diameter of 10mm attached with a needle with diameter of 18G (nozzle) and flat tip. The induce voltage between the nozzle and aggregation plate was 20-22kv, discharge rate was 0.1-0.5ml/h, and the gap between the tip of nozzle and aggregation plate was 13-15cm. Notable is that CS/PEO/H, CS/PEO/AV, CS/PEO/H/AV nanofibers electrospinning was done on CS/GEL/H, CS/GEL/AV, CS/GEL/H/AV thin films, respectively.

Determination of the morphological characterization of nanofibers

The morphology of nanofiber was carried out using scanning electron microscope (SEM) as an voltage of 20.0 kv (ZEISS- SIGMA, VP-500) after sputtering with gold.

Fibroblast cells culture

Human dermal fibroblast (HDF) were used for cells culture. In this study, CS/GEL/H/AV thin film with CS/PEO/H/AV nanofiber and without nanofibers due to its appropriate properties was used for in vitro cells culture test. The films with and without nanofiber were attached to the bottom of a six-compartment plate. A cell suspension of the fourth passage of fibroblast cells was prepared and after logging the count of the cells based on live and dead ones in volume unit by trypan blue dye, 5000 cells were added to each plate. The plates were then placed in an incubator (CO_2 5%;37°C) and to examine growth level and proliferation of the live cells, each part was monitored by an invert microscope (Leica-Germany) and taking photos using a digital camera (Infinity1) mounted on the microscope. To count the cells, five microscope fields with magnification of 100X were selected randomly and the number of cells in the fields was counted. This process was carried out for three times and each time four compartments were selected.

Hemolysis test

Hemolytic activities of CS/GEL/H/AV thin film was investigated with and without CS/PEO/H/AV nanofiber. Two methods were used for hemolysis test:

First method: blood agar medium was used to investigate the hemolytic activities of thin films with and without nanofibers on red blood cells. First, the samples were cut (1×1cm) and sterilized under UV light. The samples were then placed on the blood agar medium plates. All the plates were incubated at 37°C for 48 h. Then the diameters of the zones of lysis were measured.

Second method: to evaluate the percentage of hemolysis, blood samples of healthy human were poured into the anticoagulant tubes of sodium citrate to prevent blood coagulation. Then the blood with a ratio of 1: 1.25 was diluted with sodium chloride 0.9 %. The samples of thin films (1 × 1cm) were placed in the falcon tube and 0.2 ml of heparinized human blood was added to the samples. Then the volume of samples was increased up to 10 ml using sodium chloride 0.9 %. The samples centrifuged at 2500 rpm for 10 min. Afterward, the absorbance of the samples were measured at 545 nm. For negative and positive controls, sodium chloride 0.9 % and deionized water were used. Hemolysis ratio is calculated as follow:

$$HR=(D_t-D_{nc})/(D_{pc}-D_{nc})\times 100\% \quad (1)$$

where D_t, D_{nc} and D_{pc} are the absorbance of

Table 1. Preparation data of thin films. Unit: g

Thin film	CS/GEL	CS/GEL/WGO	CS/GEL/H_1	CS/GEL/H_2	CS/GEL/H_3
CS	0.5	0.5	0.5	0.5	0.5
GEL	5	5	5	5	5
Honey	0	0	5	10	20
WGO	0	2	2	2	2

Fig. 1. Photographic appearance of CS/GEL thin film

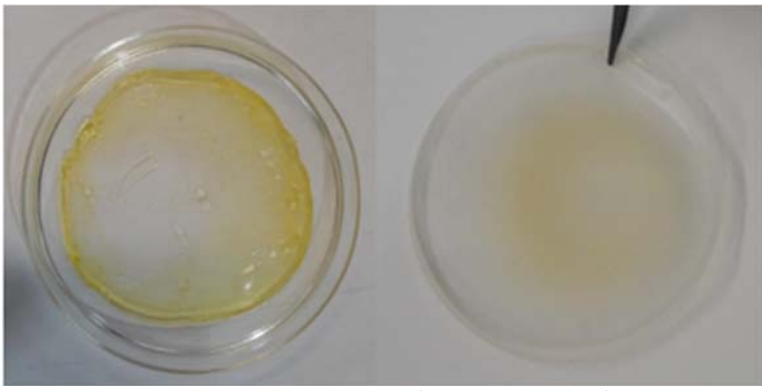

Fig. 2. Photographic appearance of CS/GEL/WGO thin film

the sample, the negative control and the positive control respectively [19].

RESULTS AND DISCUSSION

Preparation of thin films

Table 1, lists the data for thin films. Combination of CS/GEL was used for preparation of the film. The obtained film was transparent and uniform and it became wrinkled and broken into pieces in contact with water. Tensile strength and stability were also not satisfactory (Fig.1). To solve the wrinkling problem in contact with water, a low percentage of WGO (%2 v/v) was used to form a hydrophone coverage on the field. Because of emulation nature of the oil in water, the film was no longer uniform. To improve uniformity of the film, the oil was added before centrifuge step. At any rate, the tensile parameter was not satisfactory and the film was highly fragile (Fig. 2).

Fig. 3(A), illustrates CS/GEL thin films with honey (5, 10, 20% (w/v)). By adding honey, not only the new properties like more flexibility were added to the film, tensile strength was also increased. On the other hand, many biological characteristics of the film like antibacterial effect was improved. With the said ratios, no antibacterial effect was observed; still, adding 5% or 10% (w/v) honey to the film improved its flexibility and tensile strength.

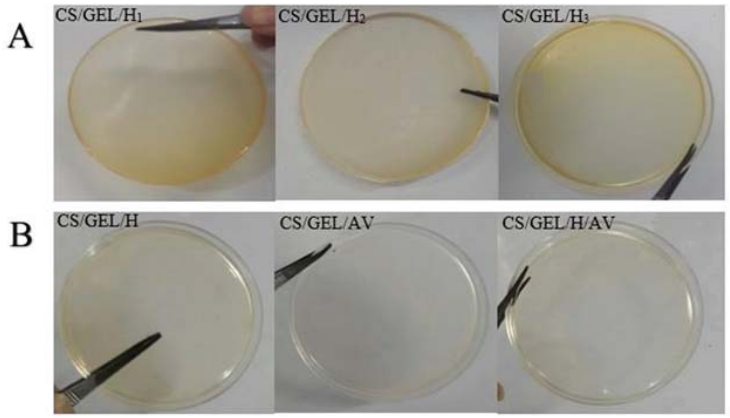

Fig. 3. Photographic appearance of (A) CS/GEL/H_1 (2% W/V), CS/GEL/H_2 (5% W/V), CS/GEL/H_3 (10% W/V) , (B) CS/GEL/H(20% W/V), CS/GEL/AV, CS/GEL/H/AV thin films

Table 2. Preparation data of thin films. Unit: g

Thin film	CS/GEL/H	CS/GEL/AV	CS/GEL/H/AV
CS	1	1	1
GEL	8	8	8
Honey	20	0	20
AV	0	20	20
WGO	2	2	2

Increase in concentration of honey up to 20% (w/v), improved its antibacterial characteristic. However, higher level of honey increased adhesiveness of the film and stability of the film was not preferable. To deal with this, the combination ratio was changed and the optimum features were obtained with GEL 8% (w/v), CS 1% (w/v), H 20% (w/v), AV 20% and WGO 2% (w/v) (Table 2).

Fig. 3(B), shows macroscopic image of CS/GEL/H, CS/GEL/AV, CS/GEL/H/AV thin films; the three films have soft, transparent, smooth, and uniform surfaces. However, the sample with honey is slightly yellowish comparing with other samples.

ATR-FTIR analysis

ATR-FTIR is the best tool to illustrate chemical variation at molecular level. Width, intensity, and position of the peaks are sensitive to chemical changes and conformation of macro-molecules.

Functional groups in the thin films were determined using AR-FTIR at 400-4000cm^{-1} range. Fig. 4, shows the ATR-FTIR spectrometry results for CS/GEL, CS/GEL/H, CS/GEL/AV and CS/GEL/H/AV films.

In CS/GEL film, the strong absorbent band at 1080cm^{-1} represents C-O stretching vibration of chitosan. In addition, the absorbent band at 1336cm^{-1} represents N-H bond of gelatin and chitosan.

The strong absorbent band at 1629cm^{-1} and 1540cm^{-1} represents peaks of the amide I and amide II, respectively. The resultant absorbent band of C-H stretching vibration in methyl groups appears at 2929 cm^{-1}.

The strong and wide absorbent band at 3292cm^{-1} represents the stretching vibration of O-H and N-H groups of chitosan and gelatin; normally, amine I demonstrates absorbent band in vicinity of 3200cm^{-1} and 3500cm^{-1} and these bands are the free N and H stretching vibrations respectively. Wider band is due to hydrogen bond between gelatin and chitosan.

The obtained spectrum of ATR-FTIR spectrometry of CS/GEL /AV showed a strong and wide band at 3289cm^{-1} region and this peak is due to the stretching vibration of O-H groups of CS, GEL, and AV groups. Moreover, this region contains the peak of stretching vibration of N-H group that overlaps with O-H group. WGO contains high level of linoleic acid with strong absorbent peak at the same region.

Additionally, the diagram features strong absorbent band at 1635cm^{-1} region, which might be due to formation of ester or amide bond between COOH, -NH_2, or -OH AV, CS, and GEL groups. At this region, CS features a peak of stretching vibration of amine group and C=O.

Absorbent band at 1558.58cm^{-1} regions is due to stretching vibration of C=C bond that indicates aloin and vinyl ether. Moreover, it indicates an aromatic ring of AV presence.

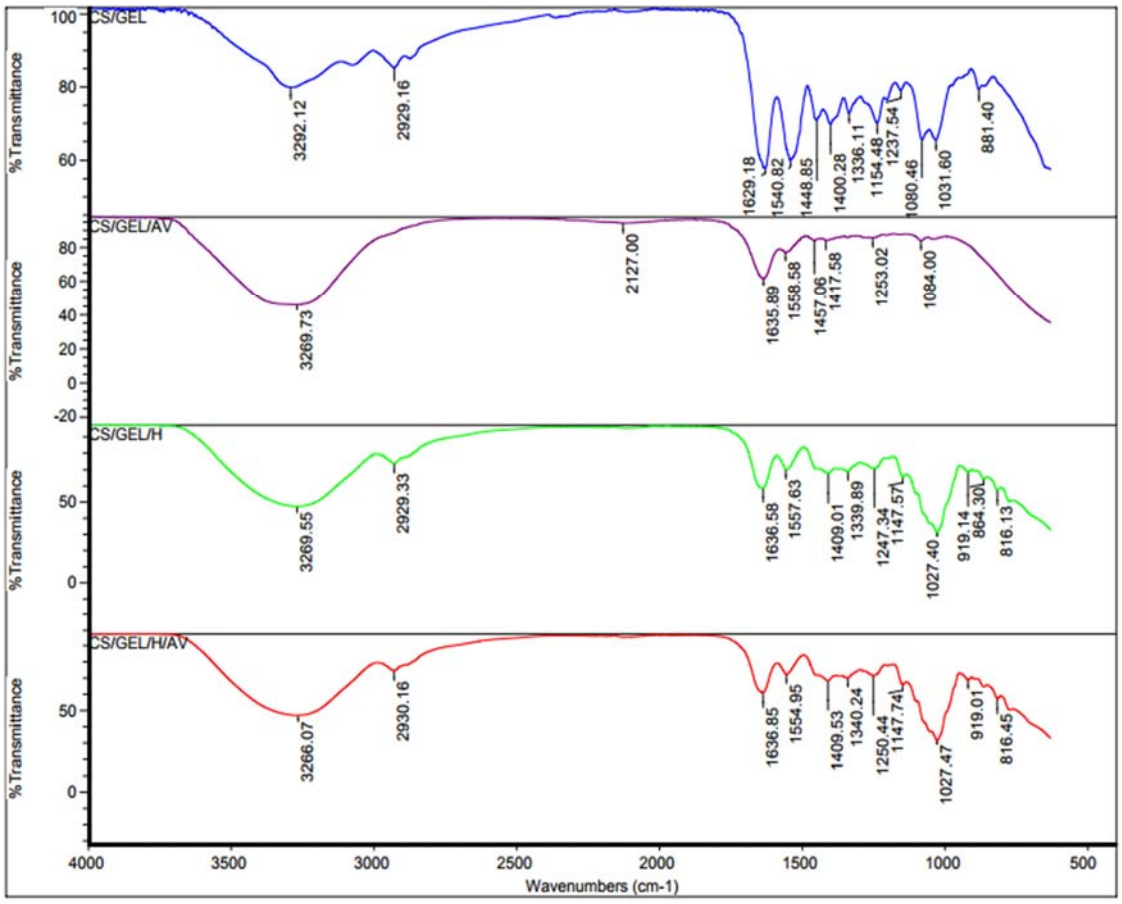

Fig. 4. ATR-FTIR spectrum of composite thin film

Absorbent band at 1240cm^{-1} represents stretching vibration of C-O group of ester and phenols. Additionally, the absorbent band at 1084cm^{-1} represents stretching vibration of C-O polysaccharides of AV.

In ATR-FTIR spectrometry of CS/GEL/H film, the absorbent band of C-H symmetrical and asymmetrical stretching vibration of CH_2 group appears at 2929cm^{-1} region, which indicates pyranose ring. The band also indicates presence of aliphatic group in the compound. Additionally, the peak indicates stretching vibration of NH_3^+ of free amino acid and C-H of carboxylic acid. Increase in the peak at this region indicates proper bond of honey compounds with CS/GEL films due to functional groups of –OH and –NH_2.

The peak observed at 1027cm^{-1} represents stretching vibration of C-O and C-C in carbohydrate structure.

In general and comparing with ATR-FTIR spectrum of CS/GEL/AV, the absorbent bands at 919cm^{-1}, 1247 cm^{-1}, and 1339 cm^{-1} confirm presence of honey in the composite.

As shown in Fig. 5, ATR-FTIR spectrum of CS/GEL/H/AV composite film is not notably different from that of CS/GEL/H film. This shows that AV is not highly effective on the interactions among CS, GEL, and honey. One reason for this can be the fact that AV was added at the final stage of preparation. Another notable point is strong interaction of honey with CS and GEL comparing with that of AV. Here, the absorbent band of 1250 cm^{-1} represents vibration of C-O phenol group of AV and absorbent band 1558 cm^{-1} represents stretching vibration of C=C bond of vinyl ether compound in AV. Each band have three units shift comparing with spectra of CS/GEL /AV thin film.

Evaluation of Antimicrobial Activity

Microbial test included measuring the size of growth inhibition halo around the films and the additives used in the different samples. Size of

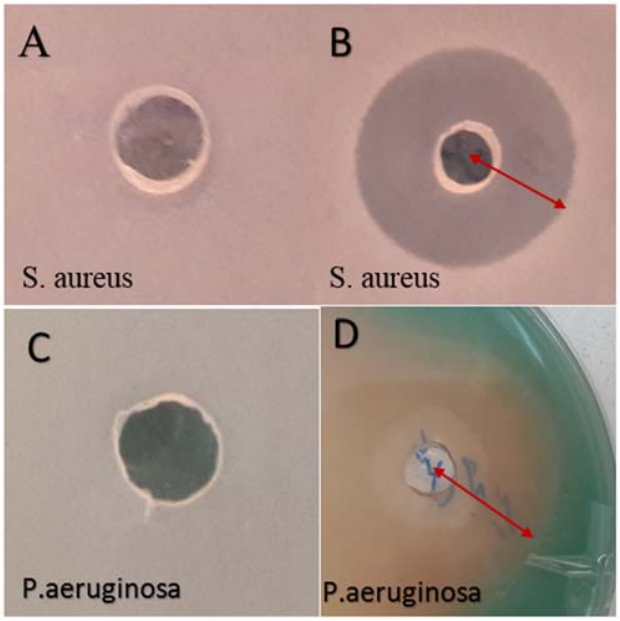

Fig. 5. The antibacterial activity of (A,C) aloe vera and (B,D) honey in the presence of S. aureus and P. aeruginosa

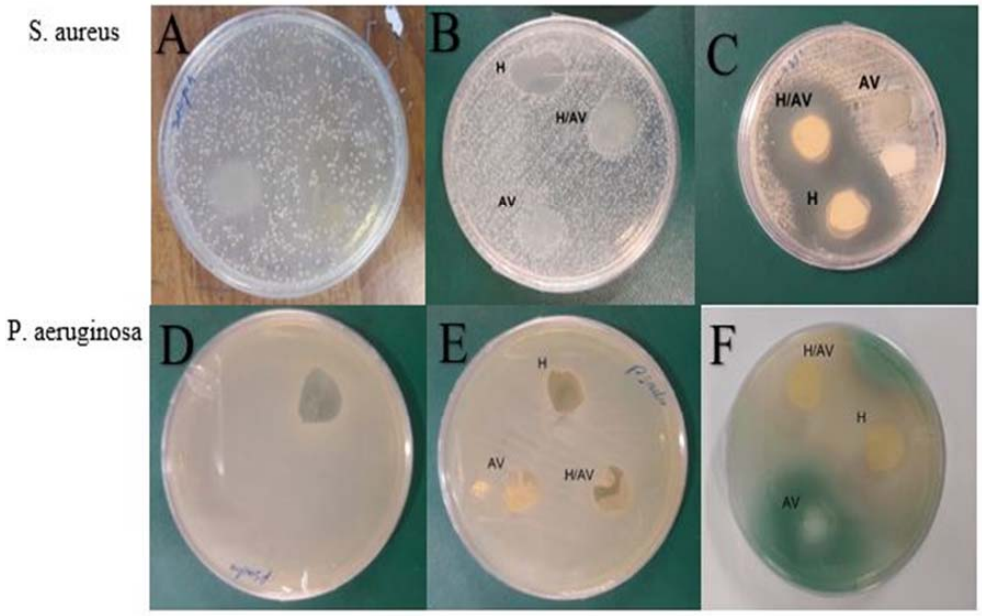

Fig. 6. Antibacterial activity of thin films

the growth inhibition halo indicates the extent of antibacterial activity that is a function of the agent effective on antibacterial activity and their release rate in agar medium.

Antibacterial activity of honey and AV against gram negative and gram positive bacteria is examined. Honey developed growth inhibition halo with 30 and 28mm diameter against *S. aureus* and *P. aeruginosa* respectively. AV gel showed no antibacterial activity (Fig. 5).

Antibacterial activity of CS/GEL film was also examined. The film showed no antibacterial activity (Figs. 6(A, D)) and one reason for this, is the limited release of the film in agar medium.

Table 3. Inhibition zone (mm) of thin films against *P. aeruginosa* and *S. aureus*

Thin film	Inhibition zone of *S. aureus*	Inhibition zone of *P. aeruginosa*
Honey	30	28
AV	0	0
CS/GEL	0	0
CS/GEL/H	26	25
CS/GEL/AV	0	0
CS/GEL/H/AV	25	25

Pereda et al. showed that the antibacterial activity of CS is not triggered without migration of the active agents. When CS is in solid state, only the organism in contact with the active site can be suppressed. Thereby, CS in its solid form cannot be released in agar medium, which means lower antibacterial activity [20]. Moreover, due to its carboxyl groups effect, GEL loses its antibacterial effects after its reaction with amine groups of CS. Lopez et al. surveyed the antimicrobial activity of CS powder against growth of gram negative bacteria and found that adding CS powder to bacterial culture was not effective on their growth. This can be explain by the low solvability of CS in physiologic pH and presence of inactive amine groups [21]. Saiz et al. showed that CS film is not an antibacterial agent against *S. aureus* and such effect can be found only in gel or solved states, where the amine groups of biopolymer are protonated (i.e. activated) [22].

Figs. 6(B, E) show the results of antibacterial activity of CS/GEL/H (H), CS/GEL/AV (AV), CS/GEL/H/AV (H/AV), thin films with concentrations of honey and AV (10% (w/v)). Clearly, at low concentration and mixed with other material, honey does not have antibacterial activity. Antibacterial activity of honey against *S. aureus* and *P. aeruginosa* were observed at concentration rate of 20%.

Based on the results, it can be concluded that CS/GEL/H/AV (H/AV) and CS/GEL/H (H) scaffolds are capable of preventing growth and proliferation of gram negative and gram positive bacteria at the infection site (Figs. 6(C, F) and Table 3).

Electrospinning of polymer solutions

Different elements were examined using electrospinning of polymer solutions without additive and eventually, 20kv voltage, 0.5ml/h discharge rate, and 14cm as the nozzle tip and surface gap were adopted as the optimum parameters for producing uniform fibers (Fig. 7(A)).

As noted, aqueous solution of CS is not suitable for electrospinning due to its high viscosity. The reason for the high viscosity of the solution is the strong hydrogen bond between NH_2 and OH groups of CS chain. To facilitate CS electrospinning, PEO was added to the solution; doing so decreases inter/intra-molecular viscosity of CS and makes the solution suitable for electrospinning. PEO molecule attaches to backbone of CS and interrupts self-aggregation of CS chain through a new hydrogen bond between -OH group of the compounds and water molecules.

There are reports in other studies that toxic solvent that kill microorganism can be used for preparing nanofibers through electrospinning. However due to toxicity of such solution, acid acetic (0.5M) was used to the extent permitted by the solubility of CS without using any additive. To obtain uniform fibers without any node, CS/PEO ratio of 70:30 was adopted. It is also possible to use CS/PEO ratio of 90:10 for electrospinning. Taking into account that honey and AV would be added to the solution and that toxic solvents have been omitted which makes electrospinning more challenging that latter ratio was not used.

As shown in SEM images (Fig. 7(B)), honey 2% creates notable nodes. Therefore, electrospinning was not employed for high concentration of honey.

Electrospinning of CS/PEO with honey, AV, and blended of them as additives with ratio of 1% w/v is shown in Figs. 7 (C,E). Formation of nanofibers though electrospinning process took place with voltage of 21kv, discharge rate of 0.1ml/h and nozzle-surface gap of 15cm.

Fibroblast cells culture

Images of fibroblast cells grown on the CS/GEL/H/AV films with CS/PEO/H/AV nanofiber and without nanofiber taken by invert microscope is pictured in Figs. 8(A, B) and percent of live cells was indicated. At the end of the second, fourth and sixth days, more than 90% of the cells are alive (Table 4).

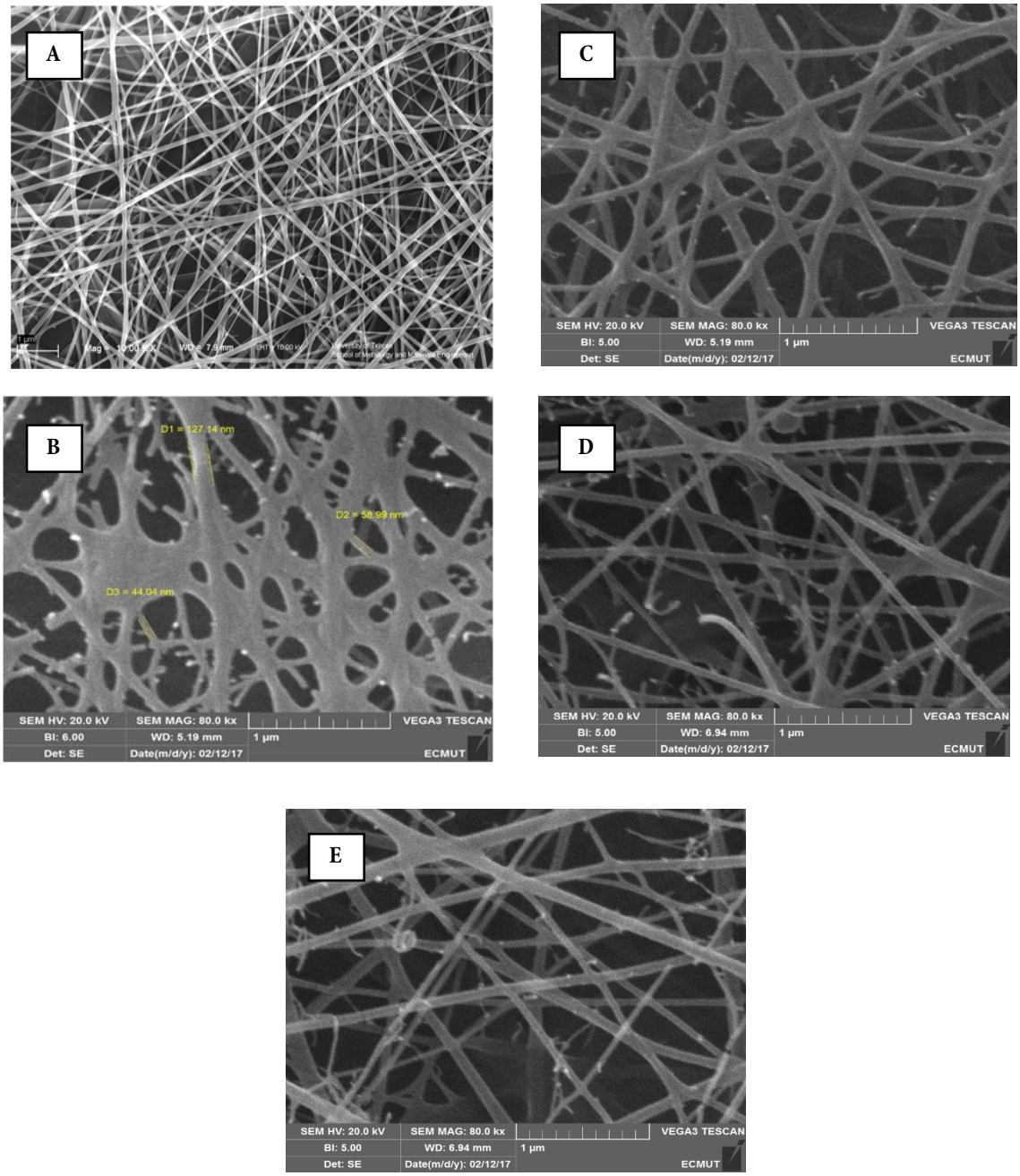

Fig. 7. SEM images of (A) CS/PEO nanofiber without additive, (B) CS/PEO nanofiber containing H 2% w/v, (C) H 1% w/v, (D) AV 1% w/v, (E) H/AV 1% w/v

Fig. 8, represent SEM images of fibroblast cells cultured on nanofiber containing films. The wide fibroblast cells appeared among the fibers are notable in the images. Clearly, the fibroblasts are grown on nanofiber containing films with acceptable physical appearance.

Fibroblast cells relatively preserved their appearance and developed wide, stretched and large. These cells were also developed among the fibers and demonstrated good adhesive feature. This indicates that the fibroblast cells have had effective interaction with the nanofibers to create a decent environment for cell growth.

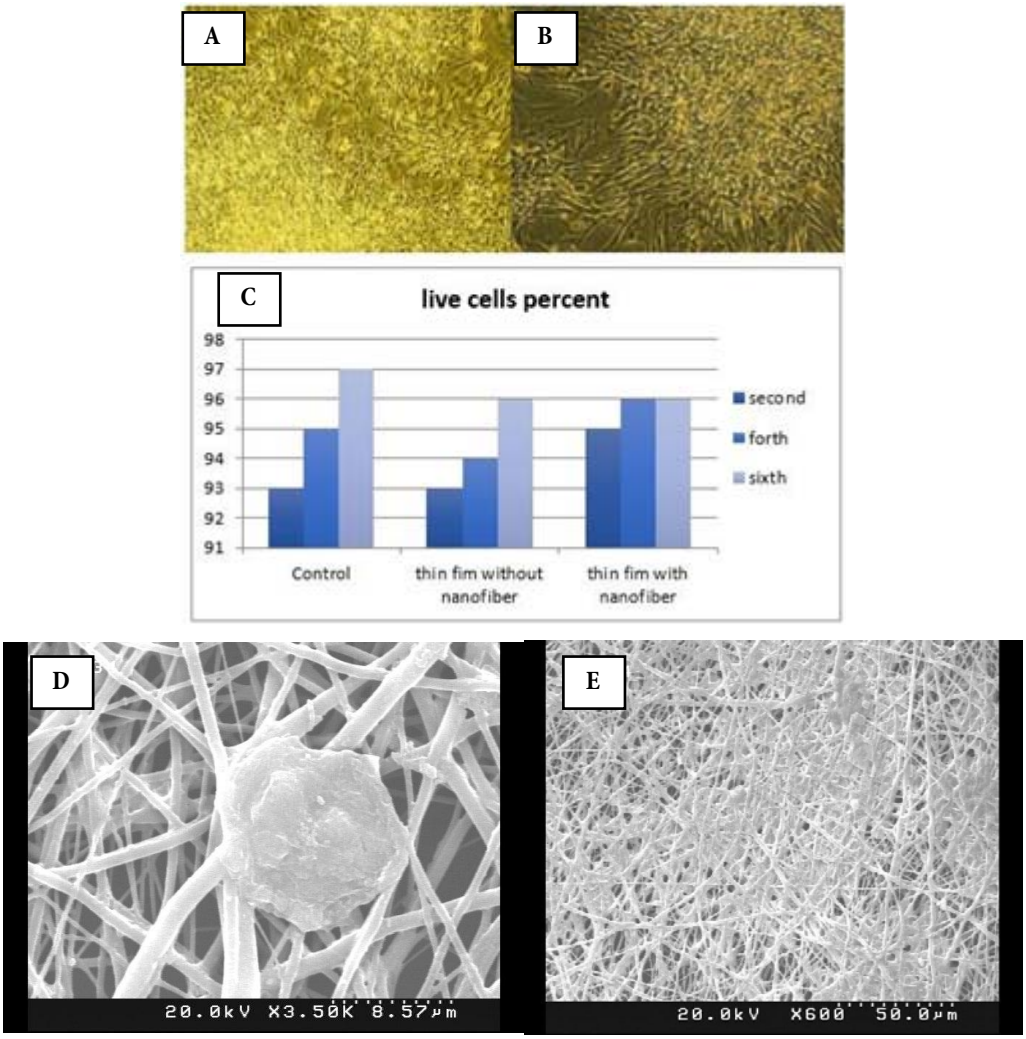

Fig. 8. Fibroblast cells in DMEM on the films (A) with and (B) without nanofiber under inverted microscope, (C) Curves of percentage of live cells after 2, 4 and 6 days, (D,E) SEM image of fibroblast cells on the film containing nanofiber

Table 4. Percentage of live fibroblast cells after 2, 4 and 6 days

Samples/Days	Second	Forth	Sixth
Control	93	95	97
Thin film without nanofiber	93	94	96
Thin film with nanofiber	95	96	96

SEM image of CS/GEL/H/AV thin film

As shown in Fig. 9, the CS/GEL/H/AV thin film was uniform and without pore and crack.

Hemolysis test

Hemolytic activities of thin films with and without nanofibers on blood agar medium are shows in Fig. 10. These results indicate that the samples are non-hemolytic.

The results represent that the hemolysis ratio of samples are well under 1%; that is similar to negative control (Fig.11) and (Table5). Therefore, the samples have an excellent hemocompatibility and can be used for medical applications.

CONCLUSION

In conclusion, the versatile thin films consisting of CS/GEL/H/AV possessed all the requisite morphological and biological properties. Thin films containing honey had antibacterial activity against *S. aureus* and *P. aeruginosa*, with significantly promoted flexibility and stretchability. Adding AV to the thin film provided mechanical

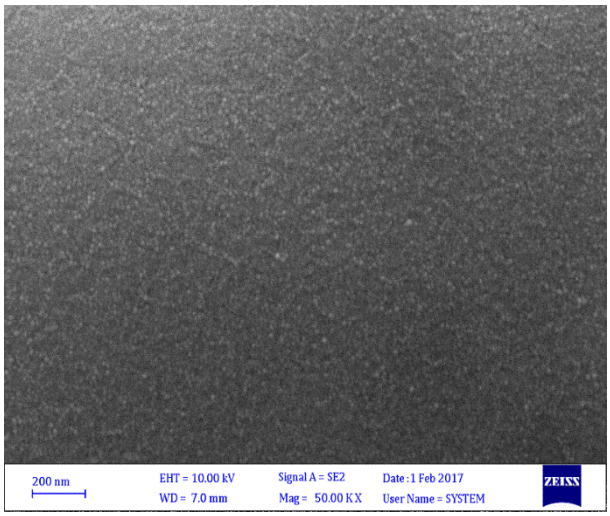

Fig. 9. SEM image of the Surface morphology of CS/GEL/H/AV thin film

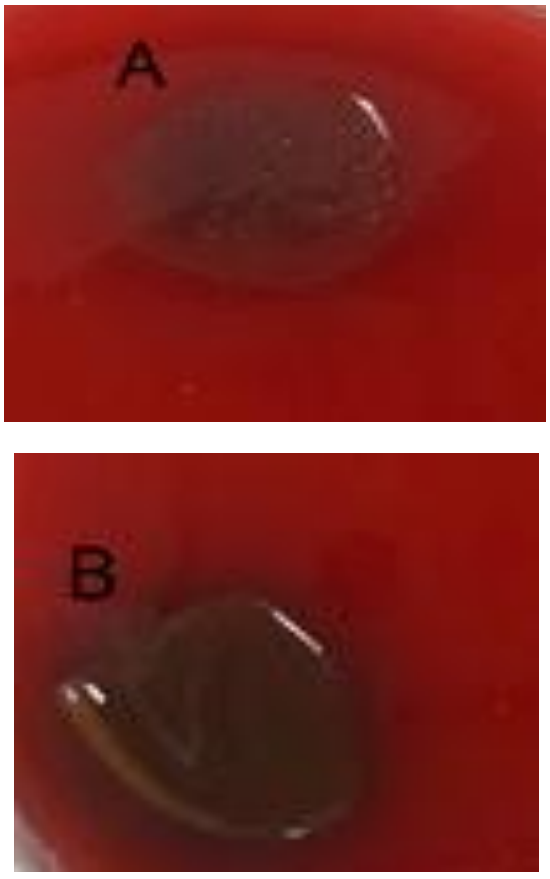

Fig. 10. Hemolysis effect of thin films (A) without and (B) with nanofiber on blood agar medium

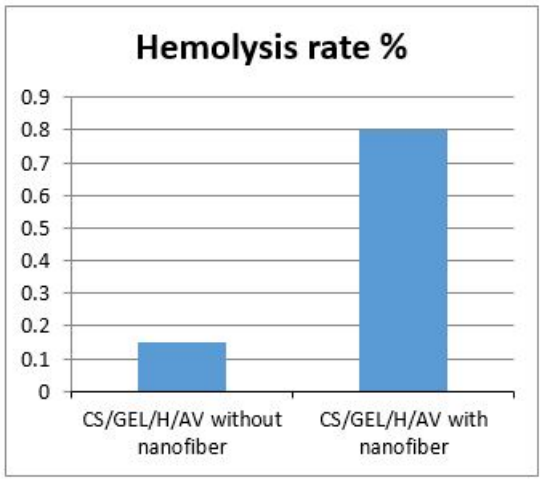

Fig. 11.Curves of hemolysis rate of thin films without and with nanofiber

strength support. This composite was non-hemolytic and supported the growth of fibroblast cells. Considering the biological properties of the thin films, they could be used as a biomaterial for treating wound, accelerating wound healing, and antibacterial coating. Moreover, the thin films were used as a base for CS/PEO nanofibers that contained the additives. Thus we can load drugs on this nanofibers for variety of purposes in medicine.

Table 5. Hemolysis ratio of the samples.

Samples	Optical density at 545 nm	Hemolysis rate (%)
Deionized water	0.751	Positive control
Sodium chloride 0.9 %	0.068	Negative control
CS/GEL/H/AV without nanofiber	0.069	0.15
CS/GEL/H/AV with nanofiber	0.076	0.8

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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