Investigation on nano microbial cellulose/honey composite for medical application

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Keywords: nano microbial cellulose (NMC), wound dressing, antibacterial property, water absorption, air permeability, thyme honey

Abstract
The overall purpose of this study is to investigate the plausibility of employing honey impregnated nano microbial cellulose (NMC) produced in Hestrin-Schramm media as a novel wound dressing. In the initial stage, three predominant characteristics of thyme, Astragalus, and Ziziphus honey including pH, total soluble solids as well as hydrogen peroxide content were assessed. In the second stage, the zone of inhibition diameters for Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were examined respectively. Meanwhile, ATR-FTIR, XRD, and SEM were applied to study the chemical, physical structures, and surface morphology of NMC pellicle. In addition, Air permeability and wettability of samples were studied. The obtained results revealed that in spite of possessing the lowest amount of hydrogen peroxide, thyme honey had the uppermost antibacterial property. Furthermore, wettability and sinking time of treated NMC with thyme honey were 43% and 49% higher than the untreated NMC respectively and purified dry raw cellulose had 11% higher air permeability than dry raw cellulose in 400 Pa. According to the results, the treated NMC with thyme honey has a high potential to be applied in the medical field as a novel wound dressing.

1. Introduction
Nano microbial cellulose (NMC) is a well-known biopolymer having diverse applications in the medical field including modern wound dressing, artificial blood vessels, drug delivery system, artificial skin as well as tissue engineering scaffold [1–6].

Considering the NMC’s notable physical and chemical properties such as high mechanical strength, high amount of water absorption, and biodegradability, it has drawn extensive attention to be the employed as one of the major biomaterials in the field of medical application [5, 7–13].

Bacteria such as Acetobacter are capable of synthesizing NMC in static culture medium as a nanofibrillar pellicle. They consume the carbon sources of the medium through the polymerization of single glucose residues into linear β-1,4-glucan chains and extracellular secretion to produce cellulose nanofibers. Eventually, assembling and crystallization of the glucan chains into hierarchically composed ribbons occur. Subsequent processes in static culture conditions lead to the formation of a three-dimensional, gelatinous structure on the surface of the liquid medium possessing an ultrafine network of cellulose nanofibres (3–8 nm) [4].

During bacterial cellulose (BC) pellicle biosynthesis on the surface of the static medium, a considerable amount of water molecules are entrapped between the forming networks of nanofibers and result in a nanofibrous hydrogel network [5].

Unique inherent properties of microbial cellulose sheets turn them into a preferential highly valued product in biotechnology. Moreover, owing to the high ability to keep the wound in a moist environment, biocompatibility as well as their potentiality to be applied in drug delivery systems, microbial cellulose as a
biodegradable hydrogel with nanofibers has been used to treat different wounds [2–4, 14–19]. However, in spite of outstanding merits, NMC does not have antibacterial activity against infection bacteria intrinsically.

Several methods are reported in the literature to induce antibacterial properties in wound dressings including utilization of antibiotics, nanoparticles [20–22], and natural products. Antibiotics have long been used as effective antibacterial agents, however, the utilization of antibiotics can intensify bacterial resistance [23, 24]. In addition, despite the proper antibacterial properties of nanoparticles, they can also diffuse into the body as a result of possessing small dimensions and impose critical health issues on patients such as deteriorating the male reproductive system [25, 26]. Among numerous drugs for wound healing with antibacterial properties, honey offers undeniable advantages and is employed for healing various types of injuries such as bedsores, burns, and wounds [27–32]. Furthermore, honey as a natural therapeutic material contributes significantly to healing different types of skin injuries by the virtue of possessing antibacterial, non-allergic, non-inflammatory as well as hemostasis enhancement properties without having hazardous effects on the human body [33–39]. The inherent bacterial infection removal characteristic of honey could be described with the presence of hydrogen peroxide produced through glucose oxidation by glucose oxidase enzyme. Moreover, antibacterial constituents, acidic pH, and a high concentration of sugar can also contribute to the antibacterial property of honey [40–42].

This research aims to use a systematical approach to assess the prominent characteristics of honey-coated NMC as novel wound dressings. Besides, morphological and structural properties of NMC were also studied.

2. Materials and methods

2.1. Materials

Three types of standard honey (Thyme, Astragalus, and Ziziphus) were supplied by Abarjes Company with the quality approval number 39/1053 from the ministry of health and medical education of Iran to be employed as NMC coating material. NMC was produced by static Hestrin-Schramm medium (glucose 2 g, peptone 0.5 g, yeast extract 0.5 g, disodium phosphate 0.27 g, and citric acid 0.115 g, to 1 liter of distilled water). 

The prepared culture mediums were sterilized by autoclaving in erlenmeyer flasks and inoculated from the solid agar culture of *Gluconacetobacter xylinus* [43]. In addition, the lyophilized form of *G. xylinus* bacteria (AATCC 23768) was obtained from the culture collection of the Iranian research organization for science and technology (Tehran, Iran).

2.2. Methods

2.2.1. NMC preparation

7-day NMC pellicles were removed from the static culture medium. Subsequently, NMC layers were purified by caustic soda 0.1 N in boiling temperature for 90 min. Afterward, purified NMC was neutralized with acetic acid. Next, the NMC was washed with tap water and later rinsed by distilled water at room temperature until reaching the neutral pH. The samples were immersed in honey for 8 h to ensure complete penetration of honey into the cellulose matrix and then were hanged to remove excess honey.

2.2.2. Characterization

2.2.2.1. Honey

In order to characterize the properties of honey; pH, total soluble solids as Brix and the concentration of hydrogen peroxide were measured by pH meter, refractometer (CODEX 2001 standard) and AOAC (association of official analytical chemists: 1990) methods respectively. 10 gr of homogenized honey and 50 ml of distilled water were added up together to determine the pH of samples. Moreover, the pH meter instrument was calibrated with the standard buffer solutions in pH 1 and an accumulation of 40 scans in range of 19–22 and pH 4, prior to measurements. Each test was repeated 5 times and the average result is reported. Also, to fully characterize the properties of different honeys, FTIR (Perkin Elmer model Frontier; USA) spectroscopy method was utilized and the results were mentioned in the manuscript.

2.2.2.2. NMC

The chemical structure of specimens was investigated by ATR-FTIR (Perkin Elmer model Frontier; USA) spectroscopy. The spectra were recorded with a resolution of 4 cm$^{-1}$ and an accumulation of 40 scans in range of 400 to 4000 cm$^{-1}$. XRD (Model: 3003PTS, SEIFER, Germany; reflection method; $\lambda = 1.540 56$ Å) was used to assess the crystallinity of samples. The crystallinity index (CI) was calculated from diffracted intensity data using the peak height of the crystalline ($I_{002}$) and amorphous regions ($I_{am}$) [4, 14].
2.3. Microscopic study
Morphology and structure of cellulose fibers were examined by applying SEM (scanning electron microscopy, XL-30 model, Philips, Poland). NMC pellets were dried and cut into small pieces (2 cm × 2 cm). After that, the samples were coated with gold before the SEM imaging step and their exposure to the electrons beam.

2.4. Wettability and air permeability
The wettability of specimens was determined by ASTM D2281-10: 2010 (Sinking time) and BS 4554: 1970 (Droplet absorption) methods respectively. In ASTM D2281-10 test NMC with distinct weight is dropped into a cylinder containing water. The time interval for the string that is connecting the weight (attached to a hook and an anchor) and NMC to relax is regarded as the sinking time. In addition, in BS 4554: 1970 method, a water droplet is placed on the sample from a specific height. The elapsed time that the droplet disappears on the sample is considered as the droplet absorption time. Moreover, BS 5636:1990 was also applied for the determination of samples air permeability. The air permeability of NMC is evaluated by applying 25 and 400 Pa air pressure difference in a surface area of 20 cm².

2.5. Antibacterial property
To determine the antibacterial property of honey by agar well diffusion method, bacteria were cultured for 24 h. Thereupon, Bacteria solute remained in physiology serum until obtaining a solution equal to 0.5 McFarland (1.5×10⁸ CFU ml⁻¹). Later on, the suspension was cultured on Agar medium (7 mm for every nuch), E. coli, AATCC 11303 (Gram-negative), and S. aureus, AATCC 6538 (Gram-positive), were the selected employed bacteria for antibacterial tests. The plates were incubated for 24 h at 37 °C under aerobic conditions. Furthermore, distinct concentrations of honey (25, 50 & 100 w/v %) were selected in order to rigorously examine the effect of induced antibacterial property as a result of honey addition to NMC.

Moreover, for accurate results, test each was done 5 times and the average of obtained values is reported. ANOVA method (α = 0.5) was utilized to analyze Standard deviation.

3. Result and discussion

3.1. Bacterial cellulose characteristic
3.1.1. ATR-FTIR
Figure 1 demonstrates the ATR-FTIR analysis of NMC. The peaks at 690 to 900 cm⁻¹ are related to C–H bonding in cellulose structure which is located in cyclohexane ring and the peaks around 1057 cm⁻¹ also confirm cyclohexane ring presence in all the resultant samples [44, 45]. The obtained peak in 1100–1200 cm⁻¹ could be attributed to the ether bond in β-glucose molecules. In addition, the peaks at 2800–3000 cm⁻¹ are ascribed to the existence of Alkane stretching bond C–H with SP3 hybridization that shows higher absorbance or they might be generally associated with aliphatic bonds in NMC [46, 47]. Furthermore, the existence of high absorbance and wide spectrum in 3200–3500 cm⁻¹ indicated the OH alcohol bond in the compound since cellulose is regarded as a polyalcohol [48, 49].

According to figure 1 all types of honey revealed similar FTIR patterns. The peaks in the range of 750–900 cm⁻¹ are related to the presence of the anomeric regions of saccharide configuration. The peak at 919.57 cm⁻¹ is attributed to the C–H bending of carbohydrates. Generally, organic acids and sugars including sucrose, glucose, and fructose reveal peaks in the range of 900–1500 cm⁻¹. The peaks at 1146 cm⁻¹ and 1256.63 cm⁻¹ is due to the C–H stretching vibration and C–O stretching of the carbohydrates content of honey. The peak at 1419.02 cm⁻¹ is a result of the O–H bending of the G–OH group as well as the C–H group of alkenes. The peak in the range of 2120–2140 might be related to the presence of nitrite groups (C≡N stretching) in honey [50–52]. The peak at 1645.32 cm⁻¹ might be assigned to the water O–H deformation. Besides, O–H stretching of water and carboxylic acids appear in the spectrum in the frequency range of 2500–2400 cm⁻¹. As a consequence, the peak at 3335.14 cm⁻¹ might be as a result of water and carboxylic acids in honey. However, this range is also where the peaks for C–H stretching vibration of carboxylic acids occur. The peak in at 2128.07 cm⁻¹ might be related to C=C stretching in honey probably as a result of the presence of chemicals such as alcohols or various monoterpenes or sesquiterpenes in honey. The peak at 2934 cm⁻¹ corresponds to the C–H stretching vibration of carboxylic acids and the NH3 stretching band of free amino acids [51–55].

3.1.2. XRD
Figure 2 illustrates the XRD pattern of purified NMC. The diffractograms reveal two main peaks in which the first peak was 2Ø = 22.7° and the second one was 14.7° for BC profile. The peaks represent the (002) and (110) lattice diffraction of polymorph and are in good agreement with the previously reported data [2, 4, 56]. Besides,
according to the XRD pattern and Segal et al approach [57], the crystallinity index of all samples was around 63% providing a favorable condition for honey absorption in the amorphous region of BC biopolymer.

3.2. The antibacterial property of Thyme, Ziziphus, and Astragalus plants

Ziziphus honey is mainly produced through the consumption of ziziphus flowers by bees, so the chemical content of its flowers is of concern. Shonouda et al [58] analyzed the Ziziphus spina-christi flower extracts. Analysis showed four main classes of chemicals including monoterpenic alcohols (mostly linalool), aldehydes (Nonanal/Hexanal), ketones (primarily 2-Undecanone) and, hydrocarbons (largely tetradecane). There were also other chemical classes such as carboxylic acids, benzene compounds, esters as well as sulfides that had a minor contribution to the chemical composition of the Ziziphus spina-christi extract. Haddouchi et al [59] characterized the essential oils of four Ruta species growing in Algeria. Results revealed that major chemical content of four tested specimens were in the 2-ketones class (2-Nonanone and 2-Undecanone). The results showed that all the essential oils had inhibition diameters of 6–17 mm against S. aureus and E. coli. Despite the
attained results, it was mentioned that ketones have antifungal activity with poor antiseptic property as well. Similar results were reported by Orlando and Nascimento [60]. 2-nonanone (C9H18O) and 2-undecanone (C11H22O) were the most abundant compounds in the Ruta graveolens L., Rutaceae essential oil. The disk sensitivity method showed that the diameter of inhibition for gram-positive (S. aureus) and gram-negative (E. coli) bacteria were 22 mm and 17.7 mm respectively. It was demonstrated that the antimicrobial activity of the essential oil was mainly due to the presence of other minor compounds that are responsible for synergistic and antagonistic interactions with main components. The antibacterial effect of aldehydes has been widely investigated. Trombetta et al [61] studied the mechanism of the antibacterial action of α, β-unsaturated aldehydes. Generally, aliphatic aldehydes perturb the lipid part of the plasmic membrane of bacteria. Despite hexanal, all the tested aldehydes (e.g. nonanal (E)-2-hexenal) made considerable changes in the permeability of cell membranes. It was demonstrated that the antibacterial activity of aldehydes had a strong relationship with the length of the alkyl hydrophobic portion of aldehydes. Moreover, saturated aldehydes such as nonanal and hexanal were less efficient in Carboxyfluorescein analysis. Although the authors claimed that aldehydes have antibacterial properties, they have also mentioned that the cytoplasmic membrane disruption is not exactly responsible for this phenomenon. There were experimental tests in which nonanal did not reveal antibacterial property despite being an aldehyde.

Two of the many well-known astragalus plants in Iran are Astragalus sahendi and Astragalus schahrdudensis [62, 63]. Movafeghi et al [63] studied the composition of volatile organic compounds in the flowers of Astragalus sahendi. Alcohols were the most abundant chemicals (the main alcohol was 2-tetradecanol). There were also many mono and sesquiterpenoid constituents such as α-terpineol, trans-geraniol, cis-geraniol, trans-geraniol and, nerolidol isomer. However, the contents of total phenols and aldehydes which contribute to the antimicrobial activity were much lower than hydrocarbons. Akhlaghi et al [62] analyzed the flowers of Astragalus schahrdudensis. The analysis showed that sesquiterpenes were the key components of flower extract. Germacrone D and Germacrone B were major components in the total extracted oil.

 Sağdaç [64] has reported the sensitivity of four pathogenic bacteria including E. coli, E. coli O157:H7, S. aureus and, Y. enterocolitica to Turkish thyme and oregano hydrosols. It is reported that all the spice hydrosols showed inhibitory effect (inhibition > 12 mm) against all bacteria. It was noted that the antibacterial effect was due to the presence of essential oils in the hydrosols. Besides, Sienkiewicz et al [65] have investigated the antibacterial activity of thyme and lavender essential oils. The components of the essential oil obtained from thyme (thymus vulgaris) were characterized. The main components were p-Cymene, γ-Terpinene, Linalool, Thymol, Carvacrol, and β-Caryophyllene. Carvacrol (cymophenol, C10H14O (OH)/(C6H5)) is a monoterpenoid phenol and thymol (2-isopropyl-5-methyl phenol, C6H3CH3(OH)(C3H7)) is a monoterpenes phenol which is an isomer of carvacrol. Scientific facts demonstrate that these biocides are capable of reducing the bacterial resistance to antibiotics with synergetic pathways [66]. Also, the cytoplasmic membrane of bacterial cells is prone to dissolve carvacrol and thymol between the lipid acyl chains since these compounds are hydrophobic [67]. There are also pieces of evidence that thymol and carvacrol decrease the intracellular ATP pool of E. coli that demonstrates the disruption of the bacterial cytoplasmic membrane [68].

p-Cymene (with two synthetic isomers of α-Cymene and m-Cymene) is a natural monoterpene. It is among the main constituents of essential oils extracted from aromatic plants through liquid extraction and steam distillation. It is demonstrated that p-Cymene can effectively induce antimicrobial effects against bacteria. However, the effect is not as significant as carvacrol. In other words, p-Cymene is not regularly used as a stand-alone bactericide. p-Cymene intensifies the effect of other antimicrobial constituents by synergism, antagonism and, additive effect mechanisms. Moreover, p-Cymene also possesses a lipolytic characteristic that prompts antimicrobial effects [69, 70]. γ-Terpinene is a lipophilic monoterpen with the chemical formula of C10H16 and two other isomers of alpha and beta terpine [71]. Oyedemi et al investigated the effect of γ-Terpinene on several gram-positive and gram-negative bacteria including L. monocytogenes, L. monocytogenes, S. pyogenes, P. vulgaris, and E. coli. γ-Terpinene revealed the highest antibacterial activity among other monoterpenes. The antibacterial effect was related to the interaction between γ-Terpinene and cell walls. While imposed with the antibacterial agent, cell walls were damaged and the result was the leakage of lipid and protein that was an implication of the bactericidal effect of γ-Terpinene [72]. β-Caryophyllene, a bicyclic sesquiterpene having a cyclobutane ring, is frequently present in essential oils of several natural plants. According to the literature, this compound has weak to moderate antimicrobial property. However, the photooxidation of β-Caryophyllene exerts improved antimicrobial activities [73, 74]. De Atenciar Filho et al [75] have also mentioned the fact that like any other lipophilic constituents in essential oils, β-Caryophyllene damages the cell walls which consequence in membrane potential reduction, proton pump collapse as well as cell lysis. Other researchers have claimed that the antimicrobial activity of β-Caryophyllene might be related to its antioxidative potential to a certain extent [76]. 3,7-dimethylocta-1,6-dien-3-ol or linalool is considered terpene alcohol with antimicrobial activity against several gram-positive and gram-negative bacteria. Terpene alcohol reveals activity against bacteria by acting as a protein denaturing, solvent and dehydrating agent [77]. Furthermore, other proposed mechanisms for the
bactericidal effect of linalool are the suppression of certain regulatory gene product translation and the inhibition of bacterial enzyme activity [78]. Despite all the mentioned research on the antimicrobial content in certain thyme plants, essential oils of local plants in different geographical regions are slightly dissimilar. For instance, some of the many folklore thyme and ziziphus plants of Iran are *Thymus daenensis* and *Ziziphus spina-christi* respectively [79]. Multiple factors such as average precipitation, average temperature, average relative humidity and, soil pH influence the final composition of the extracted essential oil. Four main components for the local thyme plant were thymol, carvacrol, γ-terpinene, and p-cymene in the research conducted by Pirbalouti et al [80]. The reported composition and main components were moderately similar to the data reported by Sienkiewicz et al for *thymus vulgaris*.

### 3.3. Effect of honey characteristics on antibacterial property

Considering the indisputable influence of Brix concentration, hydrogen peroxide content, and pH on honey properties [81, 82], each honey type (Thyme, Astragalus, and Ziziphus) was characterized separately and the calculated results are depicted in table 1. All three types of honey have acidic natures and contain hydrogen peroxide which promotes the antimicrobial properties in honey. Astragalus was the most acidic type of honey (pH = 3.68) with the highest amount of sugar (Brix=84.9) while Ziziphus possessed a H2O2 concentration of the 4.01 meq kg$^{-1}$ and had the highest amount of peroxide content. The intrinsic antimicrobial property of honey and its inhibitory ability against infection bacteria depends on the constituents of honey.

The acidic nature of honey arises from the fact that honey contains gluconic acid. There are reports that the acidic nature of honey can inhibit the biofilm formation on the wound and enhance the healing procedure by inducing more oxygen release from hemoglobin [81, 83]. In addition relatively high sugar contents of honey promote high osmolality that not only provides a moist environment by drawing the body fluid on the wound surface but also inhibits bacterial growth by hindering water to reach bacterial cell walls [84]. Although honey contains phytochemicals such as flavonoids and phenolic acids which have antibacterial properties, the major constituent that is responsible for the antibacterial property of honey is hydrogen peroxide [85]. Hydroxyl free radicals in the presence of trace metal ions can react with bacterial cell walls and nucleic acids in the cell membrane [86].

To thoroughly examine the antibacterial property, three samples with distinct concentrations of honey (25, 50 and 100 w/v %) were made and subsequently were incubated with infection bacteria (*E. coli* and *S. aureus*). Table 2 shows the zone of inhibition in 0.5 McFarland *E. coli* and *S. aureus* for 3 particular honey concentrations. The attained results signify that as a result of a decrement in thyme honey concentration, the antibacterial property of samples was drastically diminished. Furthermore, as tabulated in table 2, thyme honey had the highest antibacterial property against infection bacteria. Inhibition zone had the highest values in undiluted (100%) sample followed by a slight as well as intense reductions in lower honey concentrations for Astragalus and Thyme honey respectively. Although Ziziphus had the most amount of hydrogen peroxide content, it revealed the lowest efficiency in antibacterial tests. Given these circumstances, the higher antibacterial activity of thyme honey might be correlated with its chemical contents. A comparison between the reviewed research on the essential oils of thyme, ziziphus and, astragalus shows that thyme plant might contain

### Table 1. The properties of Thyme, Astragalus, and Ziziphus honey.

| Type of honey | pH  | BRIX (°Bx) | H$_2$O$_2$ (meq kg$^{-1}$) |
|---------------|-----|------------|--------------------------|
| Thyme         | 4.91| 84.72      | 2.3                      |
| Astragalus    | 3.68| 84.9       | 2.88                     |
| Ziziphus      | 3.98| 84.54      | 4.01                     |

### Table 2. Inhibition Zone (mm) in 0.5 McFarland *E. coli* and *S. aureus*.

| Honey Concentration (w/v %) | Type of Bacteria/Inhibition Zone (mm) |
|-----------------------------|--------------------------------------|
| 100                         | Thyme  | 9  | 18  | 8   | 11  | 7   | 10  |
| 50                          | Astragalus | 7  | 9   | 7   | 7   | 7   | 7   |
| 25                          | Ziziphus | 7  | 7   | 7   | 7   | 7   | 7   |
phytochemicals with the higher bactericidal effect. Thyme plants mainly constitute of phenolic compounds which have the highest antibacterial activity among other chemicals such as monoterpens, sesquiterpenes, aldehydes or alcohols. This might be the reason for the higher bactericidal activity of thyme honey despite possessing higher pH and lower hydrogen peroxide [77].

As demonstrated in figures 3 and 4, the inhibitory effect of thyme honey against *S. aureus* is more than *E. coli* which might be associated with the inner structure of microbe and inherent antimicrobial quality of honey. The result revealed that Astragalus was ranked as second effectual honey with antibacterial activity.

### 3.4. Evaluating the properties of treated microbial cellulose with honey

#### 3.4.1. Wettability

In wettability assessment experiments, a drop of water was placed on the specimens and the water absorption time into the wound dressing was measured latterly. Wettability tests were done to investigate the effect of purification on the wound dressing’s potential to absorb the exudate since quick exudate absorption is important for wound dressings [30]. Additionally, since the moist environment accelerates the wound healing time, the wettability of purified cellulose impregnated with honey was also evaluated.
The presented results in figure 5 indicate that the purification of cellulose marginally prolonged the timespan of the water penetration into fiber’s structure. Observations could be conceived by the elimination of hydrophilic impurities of microbial cellulose which were facilitating the water penetration into the cellulose matrix [87, 88]. Nevertheless, contrary to the dry samples, water absorption of wet purified microbial cellulose was 38.77% faster than the unpurified one. The phenomenon is because purified wet samples provide higher hydrophilic specific areas as well as surface porosity for water absorption. Furthermore, the impregnation of specimens with honey leads to an increase in surface hydrophilicity. Accordingly, the water penetration through the impregnated samples was accelerated about 120 times more than the untreated resultant samples with the wet purified layer [89].

The results of the sinking time test are shown in figure 5. Data reveals that purification and honey treatment can accelerate the speed of liquid absorption. In this test 2.5 × 2.5 cm² treated samples were put in a beaker with distilled water and the sinking time of the samples was evaluated. NMC with thyme honey had a higher sinking rate among the others. The promising measurements confirm the high potential of the treated wound dressing with honey to absorb the wound’s exudate.

As illustrated in figure 6, the purification of microbial cellulose led to an increment in the surface porosity. At the time when the dry samples are submerged in water, the trapped air in the porous sublayer of the cellulose pellicle acts as a barrier for the penetration of water molecules into the NMC matrix. Nonetheless, honey coating promotes the water absorption of NMC dramatically.

A comparison of the finding shows that the sinking rate of dry purified cellulose was 1.3 times more than the dry raw cellulose. Also, treated NMC with thyme honey had the fastest sinking time, 3.7 s, which is associated with its sugar concentration.

3.4.2. Air permeability

The air permeability of microbial cellulose was evaluated under the pressure of 25 and 400 Pa and the results are shown in table 3. Neither of dry specimens had air permeability under the pressure of 25 Pa. However, increasing the air pressure to 400 Pa, induced air permeabilities of 0.188 (ml s⁻¹.cm⁻²) and 0.209 (ml s⁻¹.cm⁻²) for the dry raw and dry purified microbial cellulose respectively. Higher air permeability in the dry purified layer is attributed to the surface impurities removal and induced higher surface layer porosity. Additionally, no air permeability was seen for the wet and treated sample with honey. The obtained results were as a result of high swelling degree and absorbed water in the porous NMC layer. The phenomenon confirms the claim that NMC sheets can inhibit the penetration of infection bacteria as well as declining the moisture evaporation on the scar surface [90–93].
3.5. SEM-microscopy

The surface morphology of NMC and the size of fibers were observed by SEM. Figure 6 shows images of NMC structure which confirm the presence of hydrophilic contaminants on the surface layer of NMC before the

Figure 6. The surface illustration of (a) contaminants on NMC, (b) Purified NMC and (c) Orientation and porosity of nanofibers.
purification step. The settlement of contaminants might be owing to the existence of various materials in the culture medium promoting more hydrophilic properties of unpurified cellulose. Figures 6(a) and (b) also illustrates the trapped bacteria and extra contaminants between nanofibers. In addition, purification with sodium hydroxide (NaOH) changes the surface property of the NMC layer by contaminant exclusion [87]. Therefore, as shown in figure 6(c), unlike the untreated NMC the purified NMC had higher porosity. The diameters of fibers were varied between 30–100 nm which enhances the special surface and caused an increment in moisture and exudate absorption. Furthermore, higher porosity of NMC increases the moisture content of NMC which can speed up the wound’s healing time. It is worth mentioning that the purification step intensified the transparency of NMC making it easier to examine the wound (figure 7).

4. Conclusion

NMC and honey have a significant role in wound healing and have been applied to different types of injuries separately. In this research, NMC was employed as an effective biopolymer to treat the wound and honey was used as a useful traditional therapeutic material to evaluate the potential of this combination as a modern wound dressing.

According to the obtained results for the treated layer with three types of honey (Thyme, Astragalus, and Ziziphus) indicated that the treated layer can absorb water 120 times faster than purified microbial cellulose. They also can collect exudate from surface 1.37 times faster which can accelerate the rate of treatment via retaining the moisture in the wound. NMC/honey did not show any air permeability in 400 Pa which is related to the filled porosity of samples. This property has the ability to retain moisture at the wound surface and prevents harmful bacteria penetration to the wound. Among all three kinds of honey, thyme has shown the highest antibacterial properties despite having fewer amounts of hydrogen peroxide and higher pH. Accordingly, the antibacterial property of thyme honey is related to inherent antibacterial material. Considering the obtained results, the NMC with thyme honey has a high potential to be applied as a modern wound dressing.
Acknowledgments

Financial support for this project was provided by the Islamic Azad University, South Tehran Branch.

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