Nanomed Res J 5(2): 192-201, Spring 2020

RESEARCH ARTICLE

Promising antibacterial activity of a mat of polycaprolactone nanofibers impregnated with a green nanogel

Abbas Abdollahi[1], Elham Zarenezhad[2], Mahmoud Osanloo[3,1*], Ghazal Ghaznavi[4], Marzieh Khalili pour[5]

[1] Department of Microbiology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran.

[2] Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran.

[3] Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran.

[4] Department of Medical Biotechnology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran

[5] Student Research Committee, Fasa University of Medical Sciences, Fasa, Iran

ARTICLE INFO

Article History:
Received 04 March 2020
Accepted 13 April 2020
Published 15 May 2020

Keywords:
Citrus sinensis
Essential oil
PCL nanofibers
Electrospinning
Nanogel
Antibacterial activity

ABSTRACT

Skin is the body's first defense line against environmental pathogens. However, impaired skin functions as a result of open wounds can cause to the entrance of opportunistic bacteria to the body. Recently, the development of nano-dressing containing green antibiotics has been received much attention around the world. In this study, the essential oil of *Citrus sinensis* (CSEO) was used as an antibacterial agent. The ingredients of CSEO were identified by GC-MS analysis with five major components of Limonene (61.83%), trans-p-2,8-Menthadien-1-ol (4.95%), trans-Limonene oxide (2.29 %), Cis- Limonene oxide (2.58 %), and trans-Carveol (2.90%). Nanogel of CSEO was prepared by the addition of a gelling agent (carbomer 940 1.5%) to its optimum nanoemulsion with a particle size of 125 ± 4 nm. Also, electrospun nanofibers of polycaprolactone with a mean diameter of 186 ± 36 nm were prepared. Characterization of the nanofibers, including SEM, ATR-FTIR, and contact-angle measurement, were carried out. After that, the nanogel was impregnated on the surface of the nanofibers, NGelNFs. Interestingly, NGelNFs completely inhibited the growth (~ 0%) of four important human bacteria strains, including *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus aureus.*

The prepared prototype, NGelNFs, can be used as a potent antibacterial agent. Furthermore, this work introduced an effective and new method for the preparation of green antibacterial agents as well as antibiotic-free wound dressings.

How to cite this article
Abdollahi A, Zarenezhad E, Osanloo M, GhaznaviGh, Khalili pour M. Promising antibacterial activity of a mat of polycaprolactone nanofibers impregnated with a green nanogel. Nanomed Res J, 2020; 5(2): 192-201.
DOI: 10.22034/nmrj.2020.02.010

INTRODUCTION

The largest organ of the human, with about one-sixth of the total body weight, is skin. It involved in many functions such as sensation, thermoregulation, and immune functions [1]. Due to the large surface of the skin, it is likely to be injured. Skin wounds are introducing as any interruption in the continuity of the skin. They are caused by many types of stress, including physical, chemical, and mechanical trauma, or triggered by a medical condition (diabetes). Nevertheless, all open skin wounds are colonized with bacteria; this can lead to an extension of the wound healing time [2, 3].

Human skin is covered with microflora; it inhibits the growth of pathogenic bacteria through

* Corresponding Author Email: m.osanloo@fums.ac.ir
osanloo_mahmood@yahoo.com

bacterial interference, nutrient competition, as well as producing metabolites that inhibit pathogen growth [4, 5]. Besides, phagocytosis cells such as neutrophils and macrophages in chronic or open skin wounds have a crucial role in the control of bacterial infections [6, 7].

On the other hand, many opportunistic bacteria from gram-positive or -negative such as *Escherichia* coli, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and others, can enter the wounds [8]. It caused a complicated infection, especially in humans, with a weak immune system [9, 10]. The emergence of microbial resistance and high load of pathogens in the environment has led to many challenges in the treatment of skin wounds; unfortunately, sometimes treatment with systemic antibiotics are advised [11, 12].

Nanofibers (NF)s containing green antibacterial agents, such as plant-derived essential oils, are introduces as a new generation of dressings that able to cure bacterial infections and facilitate wound healing [13, 14]. For example, in two studies, polycaprolactone (PCL) and PCL-gelatin NFs containing Peppermint and clove essential oils were prepared, respectively; they can be reduced the growth of *S. aureus*, *E. coli* [15, 16]. PCL is a biodegradable and hydrophobic polymer from Ɛ-caprolactone, as well as has been approved by the FDA (Food and Drug Administration of the USA) [17]. It has many usages in medical applications, e.g., drug delivery, surgical suture, and wound dressing [18].

Citrus sinensis belong to family *Rutaceae* [19]. The essential oil of *C. sinensis* (CSEO) is a well-established natural antimicrobial activity against different types of pathogens, such as bacteria, fungi, or even viruses [20-22]. Therefore, it has been used in food chemistry and pharmaceutics [23].

In this study, the ingredients of CSEO were identified using GC-MS analysis. Its nanogel was then developed to facilitate topical application. Then, electrospun NFs of PCL were prepared, as dressing. Besides, the nanogel was impregnated on PCLNFs, NGelNFs. Antibacterial activity of NGelNFs was investigated against four human pathogens using ATCC100 standard method.

MATERIALS AND METHODS

Materials

Zardband Pharmaceuticals Co (Iran) provided CSEO. Tweens (20, 80), NaOH, Mueller-hinton broth, and Mueller-hinton agar were bought from Merck Chemical Co. (Germany). PCL with a mean molecular weight of 80.000 was purchased from Sigma–Aldrich (USA). *Hexafluoroisopropanol* (HFIP) and Carbomer 940 were supplied by two Indian companies, SDFCL and Suvchem, respectively.

GC-MS procedure

For identification components of CSEO, analysis of GC-MS was performed, as described in our previous study [24].

Investigation of antibacterial activity of CSEO

Antibacterial activity of CSEO was investigated against *Klebsiella pneumonia* (ATCC: 13883), *Pseudomonas aeruginosa* (ATCC: 27853), *Escherichia coli* (ATCC: 25922), and *Staphylococcus aureus* (ATCC: 25923) using 96-well plate broth microdilution [25]. For this purpose, CSEO was dissolved in normal saline (35°C) for the preparation of serial dilution in a concentration range of 16000 – 62.5 $\mu g.mL^{-1}$.

Briefly, the mentioned bacteria colonies were dissolved in Mueller-hinton broth; 1.5×10^8 CFU/mL with a turbidity of 0.5 McFarland. Eighty and twenty µL of the Mueller-hinton broth and the bacterial suspension was added to each well using an 8-channel pipette, respectively. After the addition 100 µL/well of the prepared serial dilutions, the concentration of CSEO finally fixed at 8000,4000, 2000, 1000, 500, 250, 125, 62.5, and 31.25 $\mu g.mL^{-1}$, respectively. The treated plates were then incubated for 24 h at 37°C.

Followed by, the absorption (A) of wells was read at 630 nm using a plate reader (Synergy HTX Multi-Mode Reader, USA). Growth (%) of bacteria at each concentration was determined by equation 1. Finally, inhibitory concentration 50% (IC50) CSEO against each of the bacteria strains with their lower and upper confidence limits were calculated by CalcuSyn free version (Biosoft Co. UK)

This test was performed in triplicates; in each replicate, control and blank groups were also considered. In the control groups, 80, 20, and 100 µL of the Mueller-hinton broth, bacteria suspension, and normal saline, respectively, were used. Blank wells were filled by the Mueller-hinton broth and normal saline (100: 100 µL).

$$Growth\ (\%) = (A\,sample - A\,balnk\ /\ A\,control - A\,blank)\times 100 \quad (1)$$

Preparation and characterization of nanoemulsion-based nanogel

Nanoemulsion

Fixed amounts of CSEO (125 µL) and ethanol (20 µL) and different concentrations of tween 80, tween 20, and span 80 were stirred (single or mixed) to form a homogenous solution; 500 rpm, 10 min. Followed by, distilled water was added dropwise to the homogenous solution reach to 5000 µL. The prepared mixture was stirred for another time (2000 rpm, 30 min).

The particle size and particle size distribution (SPAN) of prepared samples were carried out using the DLS type apparatus (K-One Nano, Ltd, Korea). SPAN was calculated as $D90 - D10 / D50$. Where, *D10*, *50,* and *90* are percentile of particles that have a diameter lower than these values. Optimum nanoemulsion with appropriate particle size (around 200 nm) and SPAN (< 1) was selected for the preparation of nanogel.

Nanogel dosage form

Nanogel was prepared by the addition of carbomer 940 (1.5% w/v) to the optimum nanoemulsion. First, carbomer was hydrated in a mild condition; 120 rpm, ambient temperature, overnight. After that, the pH was adjusted to 6-7 by the addition of NaOH 25% w/v for completing the gelling process.

The viscosity of the nanogel was investigated under atmospheric pressure at 25°C, by a rheometer apparatus (Anton Paar, model MCR-302, Austria). Furthermore, a blank gel was also prepared using a similar process, and the same constituents in comparison to the nanogel; only no CSEO was used.

Preparation and characterization of electrospun PCLNFs

Electrospinning

Electrospun NFs of PCL were prepared using a previously described method with slight modifications [26]. By dissolving granules of PCL in HFIP at room temperature (overnight), the PCL solution (14% w/v) was prepared. The solution was loaded into a 10 mL syringe connected to a stainless steel blunt needle (gauge 22) in an electrospinning machine (Fnm. Co. Iran). The PCL solution was injected (0.5 mL/h) using a syringe pump. The distance of the needle and rotating collector (100 rpm) was fixed at 140 mm as well as a voltage of 15 kV was applied between them. For the facilitation of the separation of formed NFs, the surface of the collector was wrapped with aluminum foil.

SEM Analysis

The morphology, diameter, and distribution diameter of the NFs were investigated using SEM analysis (TESCAN Vega3, Czech Republic). Before the observations, the samples were cut in squares pieces (1 × 1cm) for coating with gold vapors (Quorum Technologies, Q150R- ES). The diameter and size distribution of NFs was determined by Digimizer analysis software, a free version (MedCalc Software Ltd, Belgium).

ATR-FTIR

ATR-FTIR (Bruker Company, Model Tensor II) was applied to characterize functional groups of the NFs. Spectra were recorded in the range of 400–4000 cm^{-1}.

Contact angle measurement

The wettability of the surface of the NFs was evaluated by the contact angle measurement machine (Sharif Solar**,** Tehran, Iran). A 7 µL of deionized water was dropped onto the surface of the NFs. After 5 s, a photograph was gotten for investigating the hydrophobic behavior of the NFs [27].

Impregnation of the nanogel on the surface of PCLNFs

As shown in Fig.1, circular pieces of PCLNFs with a diameter of 50 mm (50 ± 5 mg) were cut from the prepared mat of PCLNFs. Both sides of them were sterilized using a UV light for 20 min. After that, 3000 mg of nanogel of CSEO was impregnated on the surface of the pieces, named NGelNFs. Furthermore, by impregnating the blank gel on PCLNFs, another sample with the name of Gel(-oil)NFs was prepared.

Antibacterial effect of NGelNFs

The antibacterial action of NGelNFs was investigated by a standard test method for texture, AATCC100, with slight modification. The new colonies of each bacteria strains were added to Mueller Hinton broth to reached 2 × 10^5 CFU/mL turbidity. After that, NGelNFs were immerged in 6 cm plates containing 5 mL of each bacteria suspension and then incubated, 24 h at 37°C. The concentration of the CSEO at each plate eventually fixed at 15000 ppm.

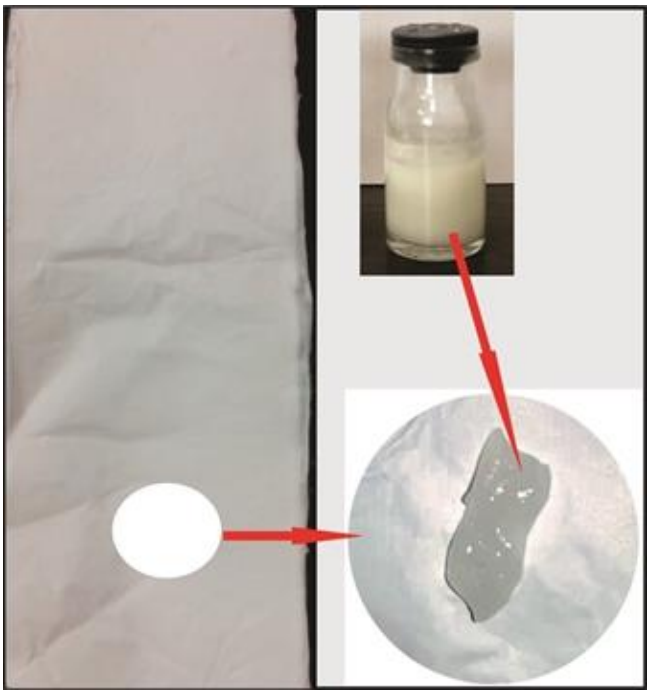


Fig. 1: Impregnating of the nanogel on the surface of PCLNFs (named NGelNFs)

After that, 10 µL of the supernatant of each plate was cultured on Mueller Hinton agar, separately and incubated for another 24 h at 37°C. Finally, the number of grown colonies on plates was counted using a colony counter. The growth reduction of each sample was calculated by equation 1. This test was performed in triplicate, in each replicates negative and control groups were considered. In negative groups, antibacterial activities of PCLNFs containing blank nanogel was examined (Gel(-oil)NFs). In the control group, no treatment was applied.

$$Growth\,reduction(\%) = (CFU\,Control(24h) - CFU\,sample(24h)/CFU\,Control(24h)) \times 100 \quad (2)$$

RESULTS AND DISCUSSIONS

Ingredients of CSEO

Thirty-two compounds were determined in CSEO using GC–MS analysis (see Table 1). Limonene (61.83%), trans-p-2,8-Menthadien-1-ol (4.95%), Limonene oxide, Trans- (2.29 %), Limonene oxide, Cis- (2.58 %), and trans-Carveol (2.90%) defined as five major ingredients.

Antibacterial activity of CSEO.

Observed IC50s with lower and upper confidence limits of CSEO against target bacteria strains are summarized in Table 2. The effectiveness (IC50) of CSEO against *S. aureus* significantly better than other (one-way ANOVA, $p < 0.05$).

Prepared nanoemulsion-based nanogel

The constituents of the prepared emulsions (26 samples) and their size analyses are listed in Table 3. For the formation of a nanoemulsion, the balance between the constituents, as well as the coordination between them, is necessary [25, 28]. For finding proper surfactants as well as the right amount, three types of typical surfactants in a single form or mixture were examined. Among the prepared samples, only No. 21 shows acceptable particle size and SPAN value; 125 ± 4 nm and 0.95 ± 0.01 (see Fig. 1A).

By the addition of carbomer 940 (1.5% w/v) to the chosen nanoemulsion (No. 21), nanogel was prepared. The effect of different shear rates on its viscosity is illustrated in Fig. 2B. The viscosity decreased by increasing of shear rate; this relation was fitted with Carreau–Yasuda model. This model is a well-known equation for non-newtonian fluids [29]. Furthermore, a blank gel was also prepared with the same process and ingredients; only no CSEO was used.

Nanogels have 3d-dimensional structures like to the biomacromolecules; thus have a wide

Table1. Chemical compositions of the CSEO using GC-MS analysis

No.	Compound	Area	%	Retention Time	Retention Index
1	Nonene	329651	0.010	8.116	
2	α-Thujene	342717	0.011	9.191	571
3	α-Pinene	37874128	1.191	9.446	613
4	Camphene	228257	0.007	10.043	623
5	Sabinene	34246642	1.077	11.148	646
6	β.-Myrcene	18060637	0.568	11.987	714
7	3-Carene	1124272	0.035	12.767	734
8	Limonene	2266978799	71.264	13.971	764
9	trans-Linalool oxide	2333414	0.073	15.729	807
10	cis-Linalool oxide	2791291	0.088	16.467	822
11	Linalool	19809530	0.623	17.08	834
12	cis-p-Menth-2,8-dienol	60702174	1.908	18.056	854
13	trans-p-2,8-Menthadien-1-ol	157669843	4.956	18.602	865
14	Limonene oxide, cis-	82287580	2.587	18.77	868
15	Limonene oxide, trans-	72968453	2.294	18.822	869
16	1,8-menthadien-4-ol	22470464	0.706	20.631	905
17	Cryptone	1481915	0.047	21.05	913
18	cis-p-Mentha-1(7),8-dien-2-ol	7476054	0.235	21.162	915
19	β. Fenchyl alcohol	4807100	0.151	21.283	917
20	Nortricyclene	31832189	1.001	21.478	921
21	8,9-epoxy-p--menth-1-ene	24869828	0.782	21.63	924
22	Prenal	23159627	0.728	21.735	926
23	trans-Carveol	92456195	2.906	22.686	944
24	Z-Citral	3670340	0.115	23.045	951
25	cis-Carveol	55583810	1.747	23.215	954
26	l-Carvone	66472215	2.090	23.734	963
27	cis-p-Mentha -2,8- dien-2-ol	8923122	0.281	27.235	1030
28	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)	27006551	0.849	28.097	1046
29	Carveol2	6185148	0.194	29.755	1078
30	4-t-Pentylcyclohexene	16817992	0.529	30.813	1098
31	trans-α-Decalone	14095234	0.443	32.39	1129
32	Valencene	16054804	0.505	34.239	1166

Table 2: Observed IC50s of CSEO against examined bacteria strains

Parameters*	*P. aeruginosa*	*K. pneumonia*	*E. coli*	*S. aureus*
IC50	4734	5764	9980	1046
LCL-*UCL	3075-7288	2965-16906	3809-26145	435-2515

*Presented as μg.mL^{-1}, **Lower Confidence Limit, ***Upper Confidence Limit

Table 3: Ingredients and size analyses of prepared CSEO emulsions

No.	CSEO	Ethanol	Tween 20	Tween 80	Span 80	Water	Particle size	SPAN
1	125	50	0	250	0	4575	21.5	13.5
2	125	50	0	300	0	4525	75.8	5.47
3	125	50	0	350	0	4475	142	1.86
4	125	50	0	400	0	4425	139	2.49
5	125	50	0	450	0	4375	295	1.71
6	125	50	0	500	0	4325	436	1.29
7	125	50	0	550	0	4275	19.5	42.7
8	125	50	0	600	0	4225	133	5.33
9	125	50	125	0	125	4575	367	0.94
10	125	50	160	0	160	4505	334	1.04
11	125	50	195	0	195	4435	336	0.96
12	125	50	230	0	230	4365	12.3	152.3
13	125	50	265	0	265	4295	257	0.96
14	125	50	300	0	300	4225	978	1.75
15	125	50	335	0	335	4155	1610	1.86
16	125	50	370	0	370	4085	12.1	367.2
17	125	50	405	0	405	4015	1500	1.97
18	125	50	440	0	440	3945	759	1.73
19	125	50	0	250	50	4525	22.5	1.87
20	125	50	0	300	60	4465	117	2
21	125	50	0	350	70	4405	125	0.95
22	125	50	0	400	80	4345	134	0.97
23	125	50	0	450	90	4285	20	1.98
24	125	50	0	500	100	4225	201	0.97
25	125	50	0	550	110	4165	161	1.52
26	125	50	0	600	120	4105	209	1.59

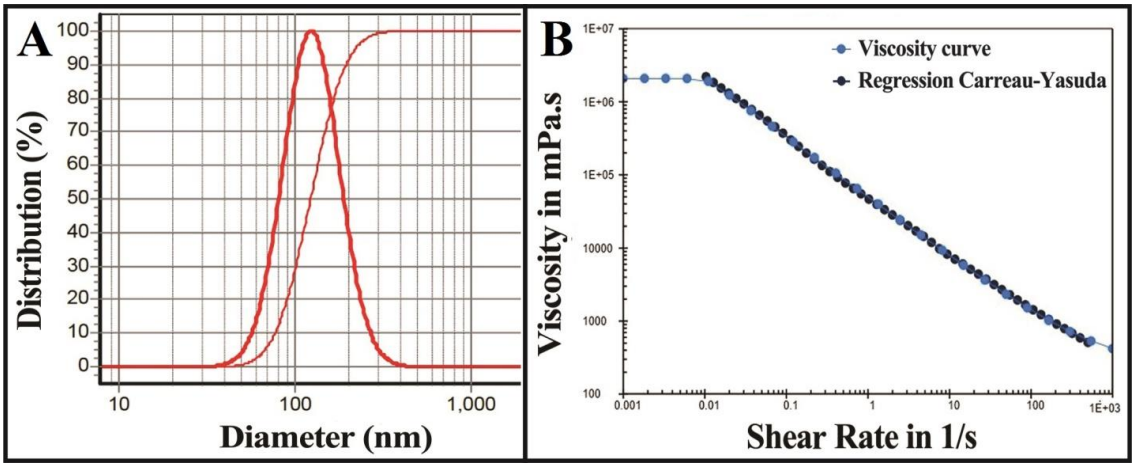
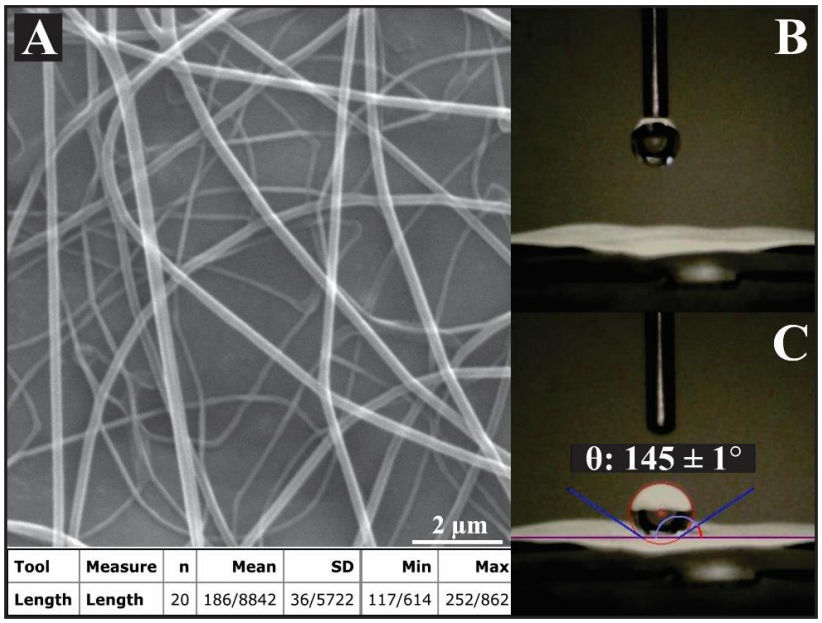


Fig. 2: A: DLS analysis of CSEO nanoemulsion, B: viscosity of the nanogel fitted with Carreau–Yasuda model.



Tool	Measure	n	Mean	SD	Min	Max
Length	Length	20	186/8842	36/5722	117/614	252/862

Fig. 3: Size and morphologies of the prepared electrospun PCLNFs (A), Water droplet during injection (B), and after 5 s (C).

range of biomedical applications [30]. Their polymeric network can be both hydrophilic and hydrophobic so they can carry all of the ionic, nonionic, and nonpolar nanoparticles [31]. Carbomers are derivatives of polyacrylic acid that used as thickening agents for the preparation of nanogels [32]. For example, topical delivery of amphotericin B was improved by preparing its nanoemulsion-based nanogel, using carbomer 980. The percutaneous permeation flux rate of nanogel (18.09±0.6 µg/cm^2/h) was better than drug solution (4.59±0.01 µg/cm^2/h) or even nanoemulsion (15.74±0.4 µg/cm^2/h) [33].

Characteristics of PCLNFs

SEM image of smooth and non-branched PCLNFs with an average size of 186 ± 36 is depicted in Fig. 3A. The contact angle of de-ionized water with the surface of PCLNFs is given in Fig. 3(B and C). The contact angle (θ) was 145 ± 1°, concluded that the surface showed superhydrophobic behavior. Noted, if the contact angle of water with any solid surface exhibits larger than 90°, it is showed hydrophobic behavior and lower values called hydrophilic [34].

The ATR-FTIR spectra of PCLNFs is displayed in Fig. 4. FTIR spectroscopy is an important identification tool for determining functional groups [35]. The absorption bands of 2943 and 2865 cm^{-1} are attributed to the C-H stretching vibration of the hydrocarbon of PCL. A strong band at 1721 cm^{-1} is related to the stretching vibration of carbonyl groups (C=O stretching of ester) of PCL. The characteristic absorption band in 1239cm^{-1} belongs to the stretching vibration of (C–O).

Antibacterial activity of NGelNFs

The antibacterial activities of PCLNFs impregnated with CSEO nanogel and blank gel (i.e., NGelNFs and Gel(-oil)NFs) are illustrated in Fig. 5. There is no significant difference seen between the effectiveness of Gel(-oil)NFs and the control group against all examined bacteria strains (Independent sample t-test, $p > 0.05$). Interestingly, the growth of all bacteria after treatment with NGelNFs reduced to ~ 0%, concluding excellent effectiveness.

Reviewing the literature, no report was found on using impregnated NFs with essential oil-based nanogel as an antibacterial agent. However, one study tried to preparing photosensitive antibacterial agents using PCLNFs and gel of silver nanoparticles. The nanoparticles were released from the nanogel after irradiation by UV light 405 nm. The released nanoparticles act as antibacterial agents against *S. aureus* and *E. coli*. However, the antibacterial test was carried out by disc diffusion; therefore, the results are not comparable with this study [36]. ATCC100 is the standard method for the investigation antibacterial effect of textures.

In another study, antibacterial properties of

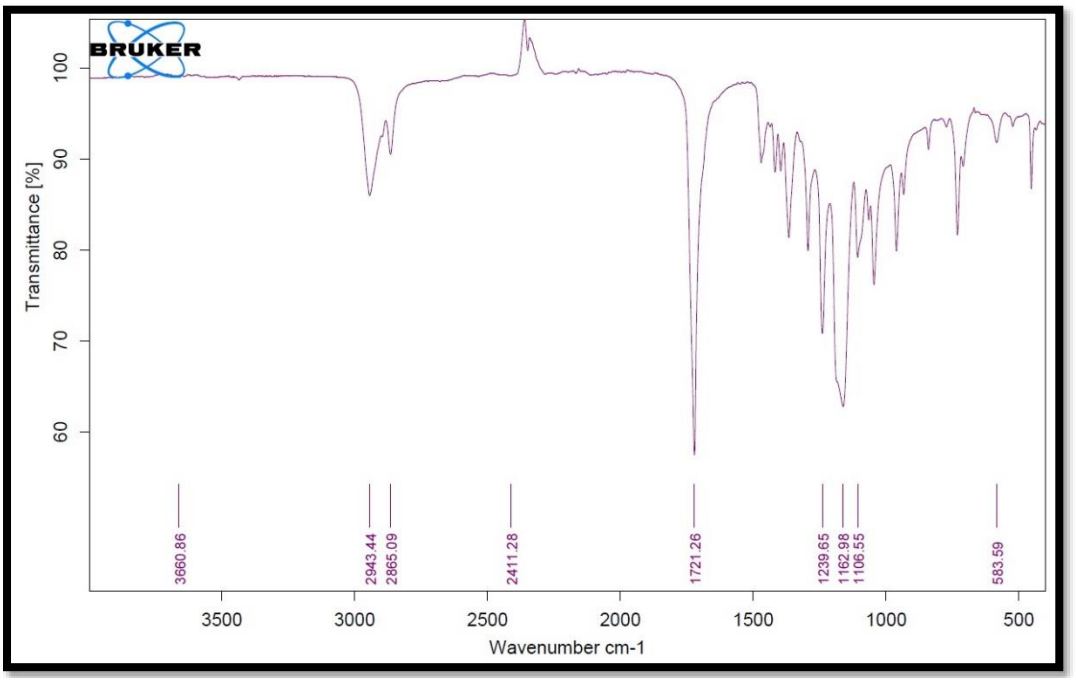


Fig. 4: ATR-FTIR spectra of PCLNFs

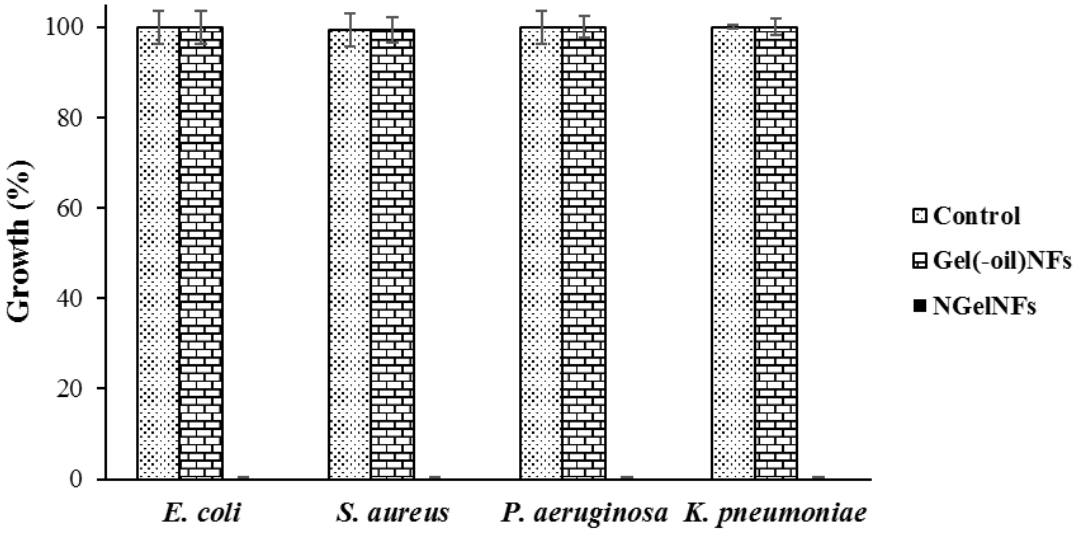


Fig. 5: Antibacterial activities of PCLNFs impregnated with CSEO nanogel and blank gel (NGelNFs and Gel(-oil)NFs)

PCLNFs mat containing Peppermint EO against *S. aureus* and *E. coli* have reported. However, the growth of examined bacteria strain reduced to 50% [37]. In addition, a nanopad of cellulose acetate containing a mixture of orange and rosemary essential oil was able to partially inhibit the growth of *E. coli* and *S. aureus* [13]

The observed results in this study are promising, completely inhibition of the growth of several important bacteria. Moreover, the prepared prototype can be used as a potent antibacterial material. However, the introduced model to make this type of antibacterial agent or antibiotic-free wound dressing is much more valuable.

In this system, by preparing nanoemulsion-based nanogel, the volatility and effectiveness of the essential oil were improved. Besides, its topical usage was facilitated. Many achievements were

also obtained by impregnating the nanogels onto the surface of the NFs. For instance, easier topical application, the possibility of packing with a certain amount of nanogels as well as preventing the entry of environmental pathogens into the wound site.

CONCLUSION

Ingredients of CSEO were identified by GC-MS analysis, and its nanoemulsion-based nanogel of CSEO was prepared. Besides, electrospun NFs of PCL were successfully prepared and impregnated with the nanogel, NGelNFs. Interestingly, the NGelNFs completely inhibited the growth of all examined bacteria strains, including *K. pneumonia*, *P. aeruginosa, E. coli*, and *S. aureus*.

The prepared prototype can be used as a potent antibacterial agent as well as the antibiotic-free type of wound dressing. Moreover, this study introduced a new approach for the preparation of nano-dressing using green constituents.

ACKNOWLEDGMENT

This study was supported by Fasa university of medical sciences, grant No. 97152. Besides, it has been ethically approved, IR.FUMS.REC.1397.142.

CONFLICT OF INTEREST

No researchers have a conflict of interest in this study.

REFERENCES

1. J. Kanitakis. Anatomy, histology and immunohistochemistry of normal human skin. Eur. J. Dermatol. 12 (4) (2002) 390-401.
2. Wysocki AB. Evaluating and Managing Open Skin Wounds: Colonization Versus Infection. AACN Clinical Issues: Advanced Practice in Acute and Critical Care. 2002;13(3):382-97.
3. Chaves MEdA, Araújo ARd, Piancastelli ACC, Pinotti M. Effects of low-power light therapy on wound healing: LASER x LED. Anais Brasileiros de Dermatologia. 2014;89(4):616-23.
4. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? British Journal of Dermatology. 2008;158(3):442-55.
5. Wang Y, Li A, Zhang L, Waqas M, Mehmood K, Iqbal M, et al. Probiotic potential of Lactobacillus on the intestinal microflora against Escherichia coli induced mice model through high-throughput sequencing. Microbial Pathogenesis. 2019;137:103760.
6. Adamson R. Role of macrophages in normal wound healing: an overview. Journal of Wound Care. 2009;18(8):349-51.
7. Osanloo M, Assadpour S, Mehravaran A, Abastabar M, Akhtari J. Niosome-loaded antifungal drugs as an effective nanocarrier system: A mini review. Current Medical Mycology. 2019.
8. Somoza-Coutiño G, Wong-Villarreal A, Blanco-González C, Pérez-Sariñana B, Mora-Herrera M, Mora-Herrera SI, et al. A bacterial strain of Pseudomonas aeruginosa B0406 pathogen opportunistic, produce a biosurfactant with tolerance to changes of pH, salinity and temperature. Microbial Pathogenesis. 2020;139:103869.
9. Johnson SA, Goddard PA, Iliffe C, Timmins B, Rickard AH, Robson G, et al. Comparative susceptibility of resident and transient hand bacteria to para-chloro-meta-xylenol and triclosan. Journal of Applied Microbiology. 2002;93(2):336-44.
10. Haghi F, Zeighami H, Monazami A, Toutouchi F, Nazaralian S, Naderi G. Diversity of virulence genes in multidrug resistant Pseudomonas aeruginosa isolated from burn wound infections. Microbial Pathogenesis. 2018;115:251-6.
11. Reuter J, Merfort I, Schempp CM. Botanicals in Dermatology. American Journal of Clinical Dermatology. 2010:1.
12. Ernst E, Pittler MH, Stevinson C. Complementary/Alternative Medicine in Dermatology. American Journal of Clinical Dermatology. 2002;3(5):341-8.
13. Liakos I, Holban A, Carzino R, Lauciello S, Grumezescu A. Electrospun Fiber Pads of Cellulose Acetate and Essential Oils with Antimicrobial Activity. Nanomaterials. 2017;7(4):84.
14. Osanloo M, Arish J, Sereshti H. Developed methods for the preparation of electrospun nanofibers containing plant-derived oil or essential oil: a systematic review. Polymer Bulletin. 2019.
15. Unalan I, Slavik B, Buettner A, Goldmann WH, Frank G, Boccaccini AR. Physical and Antibacterial Properties of Peppermint Essential Oil Loaded Poly (ε-caprolactone) (PCL) Electrospun Fiber Mats for Wound Healing. Frontiers in Bioengineering and Biotechnology. 2019;7.
16. Unalan I, Endlein SJ, Slavik B, Buettner A, Goldmann WH, Detsch R, et al. Evaluation of Electrospun Poly(ε-Caprolactone)/Gelatin Nanofiber Mats Containing Clove Essential Oil for Antibacterial Wound Dressing. Pharmaceutics. 2019;11(11):570.
17. Labet M, Thielemans W. Synthesis of polycaprolactone: a review. Chemical Society Reviews. 2009;38(12):3484.
18. Moers-Carpi MM, Sherwood S. Polycaprolactone for the Correction of Nasolabial Folds: A 24-Month, Prospective, Randomized, Controlled Clinical Trial. Dermatologic Surgery. 2013;39(3pt1):457-63.
19. Elshafie HS, Camele I. An Overview of the Biological Effects of Some Mediterranean Essential Oils on Human Health. BioMed Research International. 2017;2017:1-14.
20. P. T. Lacava, W. L. Arauújo, J. L. c. Azevedo. Evaluation of endophytic colonization of Citrus sinensis and Catharanthus roseus seedlings by endophytic bacteria. J. Microbiol. 45 (1) (2007) 11-4.
21. Sharma N, Tripathi A. Fungitoxicity of the essential oil of Citrus sinensis on post-harvest pathogens. World Journal of Microbiology and Biotechnology. 2006;22(6):587-93.
22. Muniz FR, De Souza AJ, Stipp LCL, Schinor E, Freitas W, Harakava R, et al. Genetic transformation of Citrus sinensis with Citrus tristeza virus (CTV) derived sequences and reaction of transgenic lines to CTV infection. Biologia Plantarum. 2012;56(1):162-6.
23. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food and Chemical Toxicology. 2008;46(2):446-75.
24. M. Osanloo, M. M. Sedaghat, H. Sereshti, et al., Chitosan

nanocapsules of tarragon essential oil with low cytotoxicity and long-lasting activity as a green nano-larvicide. J. Nanostruct. 9 (4) (2019) 723-35.
25. Osanloo M, Abdollahi A, Valizadeh A, Abedinpour N. Antibacterial potential of essential oils of Zataria multiflora and Mentha piperita, micro- and nano-formulated forms. Iranian Journal of Microbiology. 2020.
26. Chernonosova VS, Kvon RI, Stepanova AO, Larichev YV, Karpenko AA, Chelobanov BP, et al. Human serum albumin in electrospun PCL fibers: structure, release, and exposure on fiber surface. Polymers for Advanced Technologies. 2016;28(7):819-27.
27. Mohammadzadeh L, Rahbarghazi R, Salehi R, Mahkam M. A novel egg-shell membrane based hybrid nanofibrous scaffold for cutaneous tissue engineering. Journal of Biological Engineering. 2019;13(1).
28. Pirhadi S, Amani A. Molecular dynamics simulation of self-assembly in a nanoemulsion system. Chemical Papers. 2020;74(8):2443-8.
29. Mezger TG. The Rheology Handbook: Vincentz Network; 2019 2019/12/31.
30. Asadian-Birjand M, Sousa-Herves A, Steinhilber D, Cuggino JC, Calderon M. Functional Nanogels for Biomedical Applications. Current Medicinal Chemistry. 2012;19(29):5029-43.
31. Molina M, Asadian-Birjand M, Balach J, Bergueiro J, Miceli E, Calderón M. Stimuli-responsive nanogel composites and their application in nanomedicine. Chemical Society Reviews. 2015;44(17):6161-86.
32. Nurkeeva ZS, Khutoryanskiy VV, Mun GA, Sherbakova MV, Ivaschenko AT, Aitkhozhina NA. Polycomplexes of poly(acrylic acid) with streptomycin sulfate and their antibacterial activity. European Journal of Pharmaceutics and Biopharmaceutics. 2004;57(2):245-9.
33. Hussain A, Samad A, Singh SK, Ahsan MN, Haque MW, Faruk A, et al. Nanoemulsion gel-based topical delivery of an antifungal drug:in vitroactivity andin vivoevaluation. Drug Delivery. 2014;23(2):642-57.
34. Can MF, Avdan L, Bedeloglu AC. Properties of biodegradable PVA/sepiolite-based nanocomposite fiber mats. Polymer Composites. 2014;36(12):2334-42.
35. Faix O. Fourier Transform Infrared Spectroscopy. Methods in Lignin Chemistry: Springer Berlin Heidelberg; 1992. p. 83-109.
36. Ballesteros CAS, Correa DS, Zucolotto V. Polycaprolactone nanofiber mats decorated with photoresponsive nanogels and silver nanoparticles: Slow release for antibacterial control. Materials Science and Engineering: C. 2020;107:110334.
37. Unalan I, Slavik B, Buettner A, Goldmann WH, Frank G, Boccaccini AR. Physical and Antibacterial Properties of Peppermint Essential Oil Loaded Poly (ε-caprolactone) (PCL) Electrospun Fiber Mats for Wound Healing. Frontiers in Bioengineering and Biotechnology. 2019;7.