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Potential antibiofilm activity of farnesol-loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles against *Candida albicans*

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Abstract

Candida species are ubiquitous fungal pathogens and are the most common causes of mucosal and invasive fungal infections in humans. Especially *Candida albicans* commonly resides as a commensal in the mucosal tissues of approximately half of the human population. When the balance of the normal flora is disrupted or the immune defenses are compromised, *Candida* species can become pathogenic, often causing recurrent disease in susceptible individuals.

The treatments available for *Candida* infection are commonly drug-based and can involve topical and systemic antifungal agents. However, the use of standard antifungal therapies can be limited because of toxicity, low efficacy rates, and drug resistance. *Candida* species ability to produce drug-resistant biofilm is an important factor in human infections, because microorganisms within biofilm benefit from various advantages over their planktonic counterparts including protection from antimicrobials and chemicals. These limitations emphasize the need to develop new and more effective antifungal agents. Natural products are attractive alternatives for this purpose due to their broad spectrum of biological activities. Farnesol is produced by many microorganisms and found in some essential oils. It has also a great attention as a quorum-sensing molecule and virulence factor. It has also antimicrobial potential due to its inhibitory effects on various bacteria and fungi. However, as it is a hydrophobic component, its solubility and biofilm inhibiting properties are limited.

To overcome these shortcomings, nanoparticle-based drug delivery systems have been successfully used. For this purpose, especially using biodegradable polymeric nanoparticles has gained increasing attention owing to their biocompatibility and minimal toxicity. Poly (DL-lactide-co-glycolide) (PLGA) is the most widely used polymer in this area. In this study, farnesol is loaded to PLGA nanoparticles (F-PLGA NPs) by emulsion evaporation method and characterized by DLS, TEM, and FT-IR analyses. Our TEM findings indicate that the sizes of F-PLGA NPs are approximately 140 nm. The effects of F-PLGA NPs on planktonic cells and biofilm formation of *C. albicans* were compared with effects of farnesol alone. Farnesol inhibits the growth at a range of 53% at a concentration of 2.5 μ L compared to the control group. This rate is 45% for F-PLGA NPs at the same concentration. However, although farnesol amount in F-PLGA is approximately 22.5% of the total volume, the observed effect is significant. In TEM examinations, planktonic *Candida* cells treated with farnesol showed relatively regular ultrastructural morphology. Few membrane and wall damage and electron density in the cytoplasm were determined. In F-PLGA NP-treated cells, increased irregular cell morphology, membrane and wall damages, and large vacuoles are observed. Our SEM and XTT data suggest that F-PLGA NPs can reduce the biofilm formation at lower concentrations than farnesol

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alone 57%, and our results showed that F-PLGA NPs are effective and biocompatible alternatives for inhibiting growth and biofilm formation of *C. albicans*, but detailed studies are needed.

Keywords: Biofilm, *Candida*, Farnesol, PLGA, Nanoparticle

Introduction

Candida species are usually yeasts that can colonize the skin and mucous membranes; however, they may cause both widespread mucosal infections and serious life-threatening systemic diseases in immunosuppressed individuals (Laihadı et al. 2017). *C. albicans* is the most commonly reported agent of invasive candidiasis (Oz et al. 2013). In addition to virulence factors such as germ tube and pseudohyphae production, phenotypic change, phospholipase enzyme, secreted aspartyl proteinases, and the host immune system play an important role in the development of infection. Biofilm production is also another important virulence factor of *Candida* species, and more than 80% of human infections are estimated to be biofilm related. Cells in biofilm are much more resistant to host defense mechanisms, phagocytosis, biocides, and antibiotic treatment than their planktonics (Dag et al. 2014).

Recently, *Candida* infections show an increasing resistance to fluconazole and amphotericin B, and biofilm-producing *Candida* species are associated with a higher mortality (Rajendran et al. 2016). Existing drugs are insufficient for biofilm treatment, and they need to be administered at higher doses and more frequently. This leads to significant side effects and toxicity. In recent years, the use of natural compounds against microorganisms resistant to conventional antibiotic and antifungal therapy has been an interesting alternative. These compounds are important because of their low cost, biocompatibility, and potential antibiotic properties. Furthermore, the resistance of microorganisms to natural compounds has not been reported to date.

Farnesol is produced as a by product of the ergosterol biosynthesis pathway, and *C. albicans* produces high amounts of farnesol in dense cultures (Nickerson et al. 2006). The most significant effect of farnesol is that it has an inhibitory effect on yeast-hyphae transition at levels greater or equal to 300 μ M (Hornby et al. 2001; Costa et al. 2019). On the other hand, it is thought to be involved in the regulation of yeast cells spreading from mature biofilm surface (Lindsay et al. 2012). It is also stated that farnesol has inhibitory effects in the initial adhesion stage of biofilm formation (Cao et al. 2005). Although there is no significant effect on biofilm maturation, single cells in the mature biofilm may become susceptible to farnesol again. Farnesol destroys the cell membrane in bacteria and affects cell-membrane

functions by increasing proton permeability (Jeon et al. 2011). However, due to the hydrophobic properties of farnesol, its solubility is limited and its biofilm effect is poor (Rowat et al. 2005). For example, in order to inhibit the development of oral biofilms, it should be used in high concentrations and with multiple treatment strategies (Sims et al. 2019).

In recent studies, various drugs and natural compounds are loaded on nanovectors to increase the efficacy of the active substance and reduce the amount of used antifungal. The antimicrobial activity of natural compounds combined with the unique properties of nanoparticles and functional surfaces can be obtained (Ozturk et al. 2020). Especially poly (lactide-co-glycolide) (PLGA), a synthetic polymer, is a carrier with very promising results (Landis et al. 2016). It is biocompatible and biodegradable and approved by the Food and Drug Administration (FDA). PLGA is also the most widely accepted biodegradable polymer available. The aim of this study was to investigate the effects of PLGA nanoparticles arrested with farnesol on *C. albicans* biofilm. With the nanoparticle synthesized, it was aimed both to improve the low solubility of farnesol in water and to increase its antimicrobial and antibiofilm effectiveness on *C. albicans* planktonic and biofilm cells and to compare it with the nanoparticle-free activities.

Materials and methods

Materials

PLGA (Mw 40,000 to 75,000 g/mol), polyvinyl alcohol (98% to 99% hydrolysis degree and average Mw 30,000 to 50,000 g/mol), and farnesol (3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol) (%95) were purchased from Sigma-Aldrich.

Microorganism

For antimicrobial and antibiofilm activity studies, *C. albicans* ATCC 14053 standard strain was used. It was cultured on Yeast Peptone Dextrose (YPD) medium (Sigma-Aldrich) and RPMI 1640 (with L-glutamine, Sigma-Aldrich) broth at 37 °C for 24 h.

Synthesis of farnesol-loaded PLGA nanoparticles

Both unloaded PLGA nanoparticles and F-PLGA NPs were formed using the emulsion evaporation method similar to the method outlined previously (Gomes et al. 2011). For organic phase constitution, 50 mg of PLGA

dissolved in 2 mL dichloromethane with or without farnesol (22.5% (w/w) was prepared. The aqueous phase (20 mL) was created with 0.3% (w/v) polyvinyl alcohol (PVA) in nanopure water. The organic phase added drop-wise to the aqueous phase. Then, this mixture was homogenized for 2 min at 9500 rpm using an Ultra Turrax T25 basic. This emulsion was sonicated in an ice bath at 2 °C and 70 W of energy output during 10 min. The organic phase (dichloromethane) was removed for 20 min using a rotary evaporator. The same procedure was used for the preparation of unloaded control nanoparticles, but farnesol was not added to the organic solvent. After synthesis, the nanoparticles were purified by dialysis to remove the excess of PVA and farnesol. The nanoparticles were ultrafiltered with 200 mL of water and 50 mL was collected. Then, samples were lyophilized and stored in a desiccator until further use (Gomes et al. 2011).

Particle size and morphology characterization

Particle size and size distribution of the nanoparticles were measured by using Zetasizer Nano Series (Nano-ZS). Morphological characterization was performed using transmission electron microscopy (TEM) (Hitachi HT 7800, Japan) at an accelerating voltage of 100 kV. For analysis, sample was dropped on a copper grid and was examined after it is well dried under the TEM.

FT-IR spectra

FT-IR studies were carried out on Perkin unit (PerkinElmer Spectrum Two). PLGA nanoparticles and F-PLGA NPs were thoroughly washed three times with distilled water prior to the FT-IR experiments to remove organic compounds that were not bound to the nanoparticle surfaces. The diamond ATR technique was used for obtained samples. The wavenumber sweep was 4000 to 400 cm^{-1} with a resolution of 0.4 cm^{-1} .

^1H NMR experiment

All of the proton (^1H) NMR experiments were collected with JEOL ECZ 500 R NMR spectrometer at room temperature. Proton resonance frequency was adjusted at 500.13 MHz. Deuterium dimethyl sulfoxide (d_6 -DMSO) was used as solvent.

Antifungal properties of F-PLGA NPs

In order to determine the antifungal efficacy of the farnesol and farnesol-loaded nanoparticles, it was used to the criteria proposed by Clinical and Laboratory Standards Institute procedure (CLSI M27-A2). RPMI 1640 (bicarbonate-free) (Sigma-Aldrich, Steinheim, Germany) and SDA (Difco Laboratories, USA/France) were used as culture media. They were prepared according to the manufacturer's instructions (Wayne, 2002).

The *Candida* isolates from the stock medium for fresh culture production were first incubated in RPMI 1640 liquid medium and at 37 °C for 24 h. Samples were then taken into 5 mL of 0.85% saline and the suspension turbidity adjusted to 0.5 McFarland ($1-5 \times 10^6$ cells/mL). The prepared initial suspensions were diluted 1/50 with sterile saline and then diluted 1/20 with RPMI 1640 to a concentration of $1-5 \times 10^3$ cells/mL. The broth microdilution test was performed with 96-well microplates. RPMI-1640 (Sigma, Germany) medium was used and the final concentrations were adjusted to be 2.5 $\mu\text{g/mL}$ active ingredient in vol/vol.

After incubation at 35 °C for 48 h, absorbance measurements were taken with Chromate Microplate Reader 4300. The range of MIC values of farnesol were determined as the lowest concentration ranges that could reduce fungal growth compared to the positive control.

Ultrastructural changes of planktonic *C. albicans* cells by TEM

The ultrastructural effects of farnesol and farnesol-loaded PLGA on *C. albicans* ATCC 14053 isolate were evaluated by TEM and as described previously (Ozturk et al., 2020). For this purpose, the 10 mL cell suspension was exposed to the active substance (farnesol alone and farnesol-loaded PLGA) at a concentration of 2.5 $\mu\text{g/mL}$ and then incubated at 37 °C for 48 h. The control group without active substance was also included in the study. Cell suspensions were centrifuged at 5000g for 15 min in sterile plastic centrifuge tubes and washed sequentially with PBS (phosphate-buffered saline buffer) three times (10 min each). Each sample group was prefixed in 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS) at 4 °C for 12 h. Then, they were washed three times in PBS and post-fixed for 2 h in 1% OsO_4 at room temperature. Samples were dehydrated through a graded ethanol series (30–50%, 70%, 90%, 96%, and 100%) and were polymerized at 60 °C for 48 h by embedding in epoxy resin. Ultra-thin sections of 60-nm thickness of the blocked samples were taken onto copper grids by an ultramicrotome (Leica Ultracut R). The sections were finally stained with uranyl acetate and lead citrate (Dag et al., 2012). Samples were analyzed by Hitachi HT 7800 TEM.

XTT reduction test

Antibiofilm activity was measured according to the method previously described by Ramage et al. (2001). Standardized cell suspensions (1×10^5 CFU/mL in 200 μL RPMI 1640 medium) were added 96-well flat bottomed microtiter plate. Samples were incubated at 37 °C for 1 h, and then, the wells were washed with PBS. RPMI medium containing active substance was added to the wells and the plates were incubated 24 h at 37 °C.

Colorimetric changes according to XTT reduction test results were evaluated with a microplate reader (Chromate Microplate Reader 4300) at 492–630 nm. Thus, it was aimed to investigate the effects of active substance application before biofilm formation.

Effect of the farnesol and F-PLGA on *C. albicans* biofilm formation by scanning electron microscopy (SEM)

In this study, Whatman no. 1 filter papers (6-mm diameter) were used as surface and the effect of active substances on biofilm formation (pretreatment) was analyzed with SEM (Hitachi Regulus 8230) device. For this purpose, each sterile disk with impregnated *C. albicans* containing 1×10^5 CFU/mL in RPMI 1640 medium containing the active ingredient at a concentration of %1 v/v was inoculated and incubated at 37 °C for 24 h. Filter paper disks containing microorganism without active substances served as a control, and each assay was performed in duplicate. For scanning electron microscopic (SEM) examination, samples were prefixed in 2.5 % glutaraldehyde (prepared in 0.1 M phosphate buffer, pH 7.4, for 24 h at 4 °C). Then, it was rinsed twice with PBS buffer (pH 7.4) and postfixation was performed with 1% osmium tetroxide for 1–2 h at room temperature at dark and rinsed again with PBS. Following that, the disks were dehydrated with ethyl alcohol series (30, 50, 70, 90, and 96%) for 15 min and by 100% alcohol for 30 min. Samples were dried by critical point dryer and coated with gold with a Polaron SC7620 Sputter Coater. Finally, they were examined using a scanning electron microscope (Hitachi Regulus 8230) (Dag et al. 2014).

Results

The design and synthesis of F-loaded PLGA

The illustration scheme for F-loaded PLGA is presented in Fig. 1. Synthesis procedure was performed by emulsion evaporation method.

Particle size and morphology characterization

The obtained results for the particle size, polydispersity index, and zeta potential of farnesol-loaded PLGA are shown in Fig. 2. Our TEM data show that the F-PLGA nanoparticle sizes are approximately 140 nm. Polydispersity index value was determined as 0.463 and less than 0.5. PDI value below 0.5 indicates that electrical conductivity is better, relatively. In addition, zeta potential value was determined as 658.5.

FT-IR spectra

FT-IR spectra of PLGA nanoparticles and F-PLGA nanoparticles are given in Fig. 3. Peaks observed at 3364 cm^{-1} in the spectrum of PLGA nanoparticles and 3431 cm^{-1} in the spectrum of F-PLGA nanoparticles belong to ambient humidity. OH peak of farnesol molecule was observed at 3263 cm^{-1} in the F-PLGA nanoparticles. Peaks of CH₃ groups of PLGA and F-PLGA nanoparticles were observed as 2904 and 2832 cm^{-1} , respectively. Moreover, peaks of CH₂ groups of PLGA and F-PLGA nanoparticles were observed as 2944 and 2892 cm^{-1} , respectively. The FTIR spectrum shows characteristic peaks of PLGA, for example, PLGA showed bands in the region of 1424 – 1505 cm^{-1} corresponding to CC ring stretching vibration. The absorption bands at 1193 and 1178 cm^{-1} were assigned to the CO band. Reduction in the intensities of some peaks was observed in the spectrum of F-PLGA according to the spectrum of PLGA (Fig. 3). Characteristic bands of PLGA removed significantly in the finger print region of PLGA. As a result, a change in the intensity of peaks in the range of 1000 – 1500 cm^{-1} was observed and there was a chemical interaction between farnesol and PLGA nanoparticles.

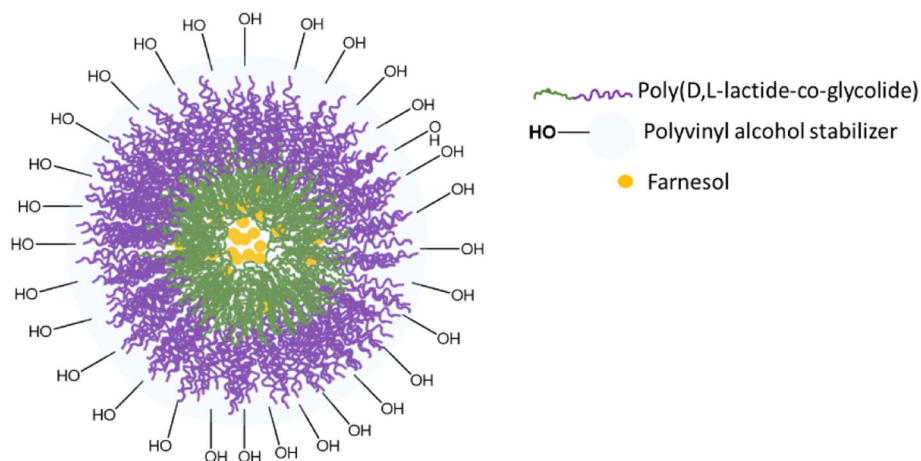


Fig. 1 Schematic illustration of farnesol-loaded particle formation

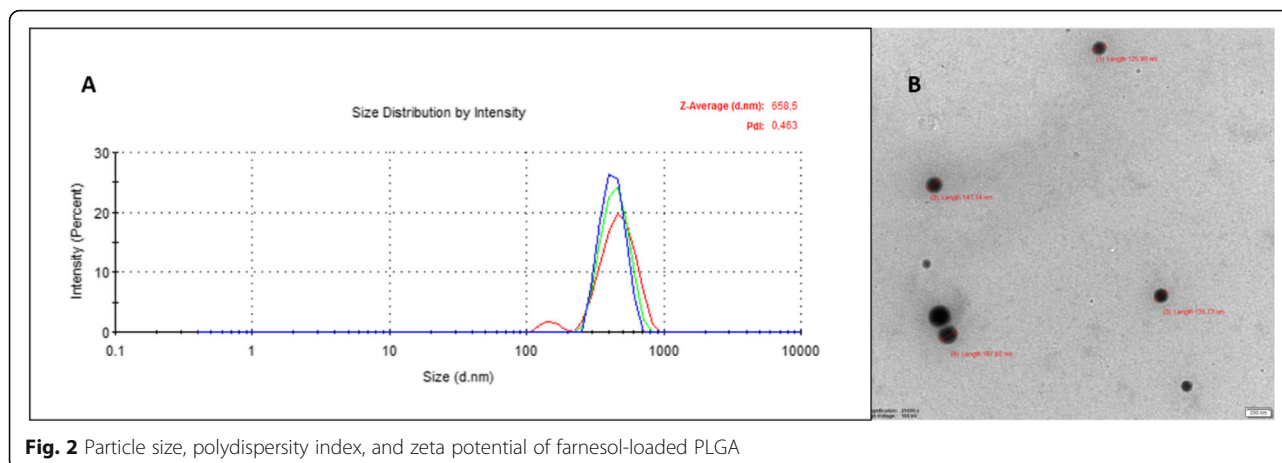


Fig. 2 Particle size, polydispersity index, and zeta potential of farnesol-loaded PLGA

¹H NMR experiment

¹H NMR spectra of farnesol and farnesol-loaded PLGA nanoparticles are given in Fig. 4. As seen in Fig. 4a, OH proton of farnesol was observed at 3.87 ppm. Moreover, methyl group's protons were seen between 1.50 and 1.60 ppm. Peaks of other protons are seen in Fig. 4a. ¹H NMR spectrum of farnesol-loaded PLGA nanoparticles is given in Fig. 4b. According to this spectrum, observed peaks in the range of 4–5 ppm belong to OH group of PVA molecule. Protons

of CH₂ groups of PVA molecule were seen between 1 and 2 ppm.

Antifungal properties of F-PLGA NPs

In our study, it was determined that methanol used to dissolve farnesol and used at 2.5% final concentration did not inhibit the growth of *C. albicans* ATCC 14053 isolate. The growth in the control group was accepted as 100%, and the reduction in MIC concentration values was given as %. According to the obtained data, farnesol

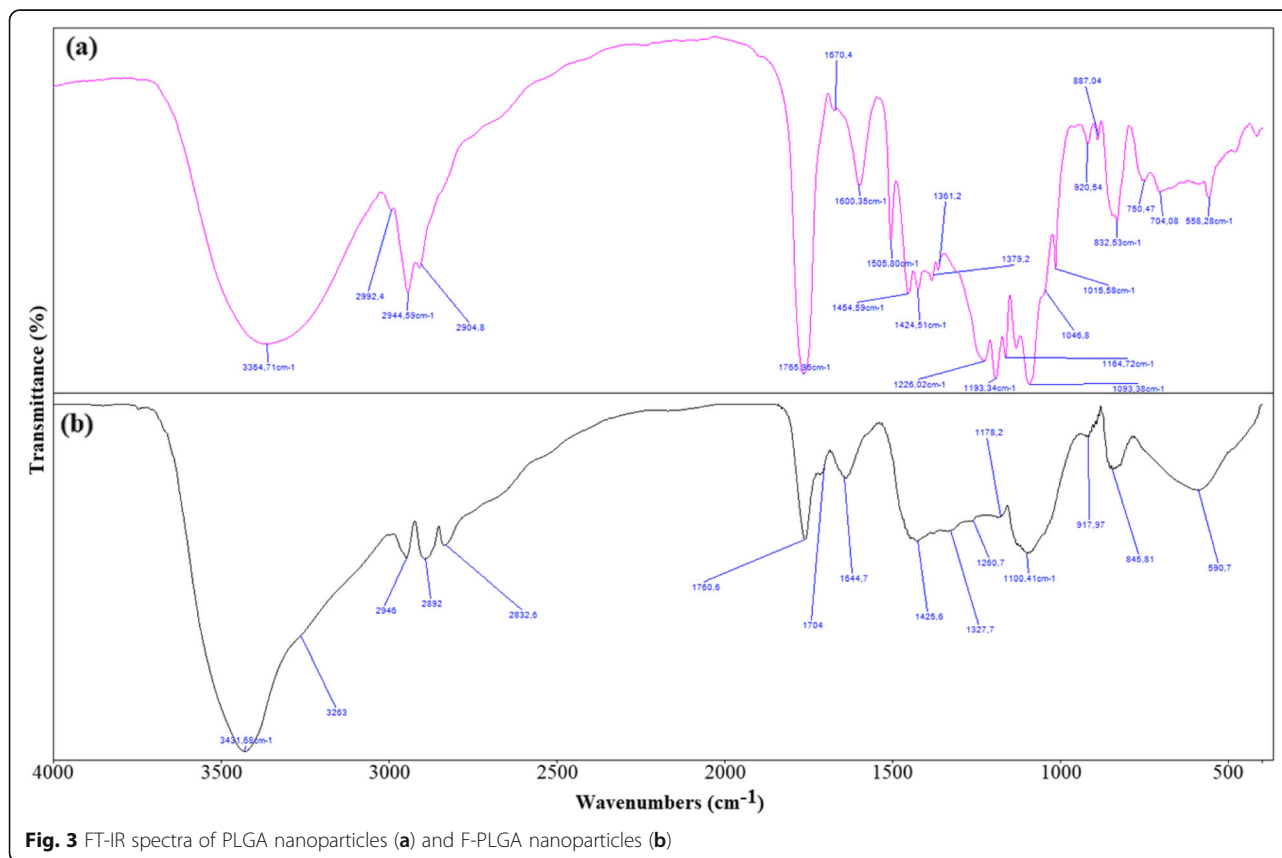
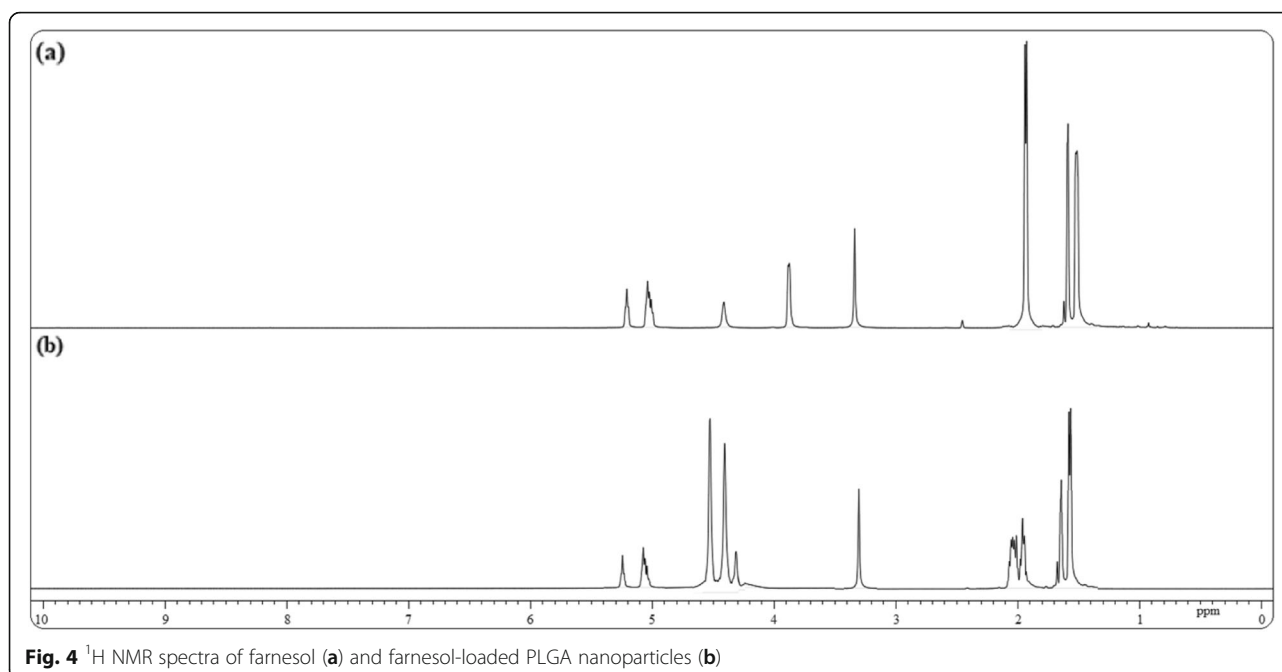


Fig. 3 FT-IR spectra of PLGA nanoparticles (a) and F-PLGA nanoparticles (b)



reduced the growth but not completely inhibited (53 to 6%) when used alone and at concentrations of 2.5 to 0.63 $\mu\text{L/mL}$. Similarly, for F-PLGA, inhibition rates were also determined based on the decrease in absorbance values at concentrations of 2.5 to 1.25 $\mu\text{L/mL}$. Reduction in growth was detected with an inhibition rate of approximately 45 to 3% (Table 1).

Ultrastructural changes of planktonic *C. albicans* cells by TEM

The effects of farnesol and F-PLGA nanoparticles on *C. albicans* ultrastructure were evaluated by TEM. As shown in Fig. 5a–c, control group cells showed well-preserved morphological features; typical and distinct cell wall-membrane structures were present. *Candida* cells treated with farnesol alone showed regular wall and membrane structures and normal oval-round structure of the cell are mostly preserved but the cytoplasm is electron dense appearance. A few cells have small vacuole formation and membrane-wall damage or rupture in the cytoplasm (Fig. 5d–g).

In cells treated with F-PLGA nanoparticles, significant increases in vacuolizations were observed and they were generally quite large and numerous. In addition, increased membrane and wall damages, occasionally lysed

cells, and generally an irregular morphology were observed (Fig. 5h–k).

Antibiofilm activity

In this study, farnesol and F-PLGA NPs were applied to the cells at the beginning (0 h) of the study and the effects of application before biofilm formation (pre-treatment) were compared. The growth in the control group was accepted as 100%, and results were compared accordingly. XTT reduction test data demonstrated the suppressive effect of both farnesol and F-PLGA NPs on biofilm formation compared to the control group. However, the effect of F-PLGA NPs with was much greater (53%) (Fig. 6).

SEM studies

Figure 7 shows SEM images of the *C. albicans* cells treated with farnesol alone and F-PLGA NPs on biofilm formation. Untreated control group *Candida* cells showed the dense biofilm and EPS matrix covered on disk surface (Fig. 7a). SEM image of the biofilm treated with farnesol alone displays that the biofilm was decreased compared with the control group (Fig. 7b). As shown in Fig. 7c, there was no significant biofilm structure observed in the F-PLGA NP-treated group; only small amounts of microorganisms could be observed.

Discussion

Farnesol is an sesquiterpene alcohol found in various plant extracts and is also a QS molecule produced by *C. albicans*. It is produced endogenously as a precursor of sterol synthesis. Due to its ability to suppress hyphae

Table 1 MIC range values observed for farnesol and F-PLGA Nps against and *Candida albicans*

Parameter	Farnesol	F-PLGA Np
MIC v/v	2.5 to 0.63 $\mu\text{L/mL}$	2.5 to 1.25 $\mu\text{L/mL}$

Values for $n = 3$; CV \leq %5

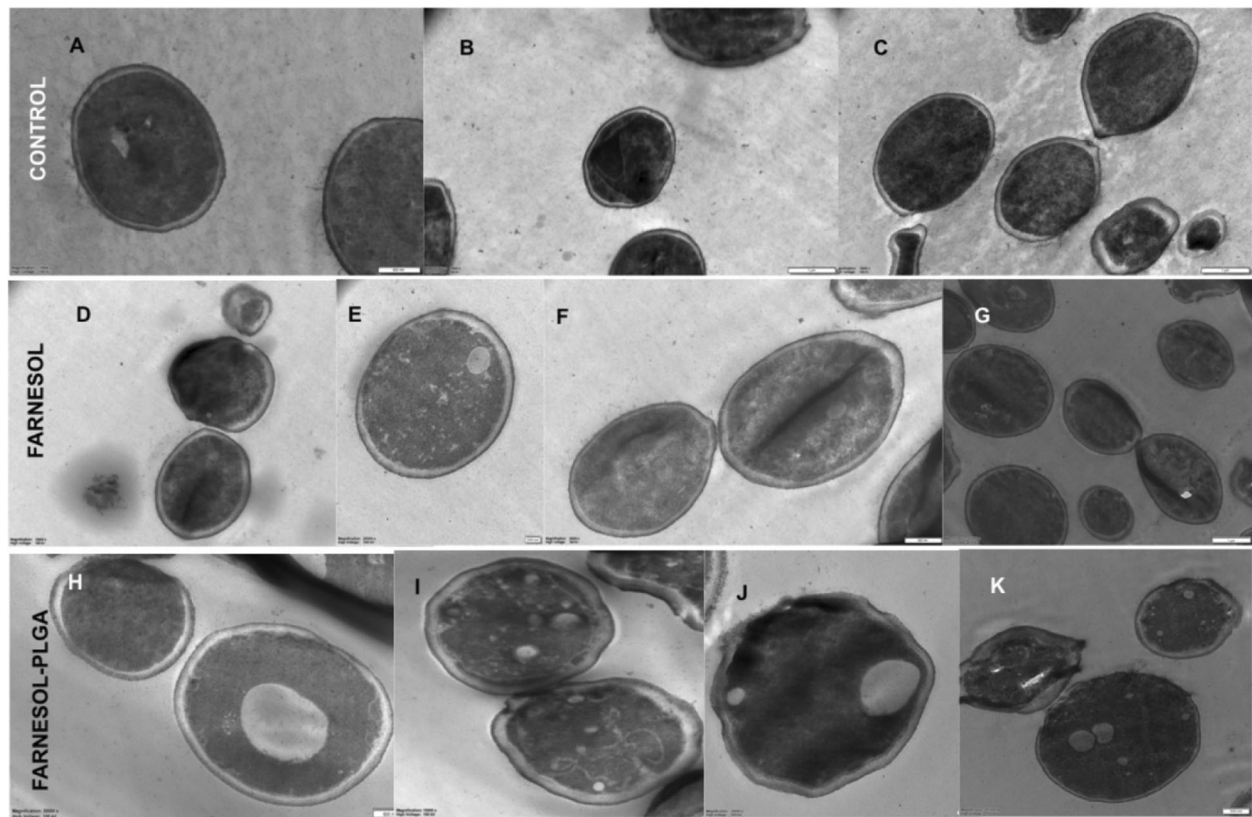


Fig. 5 TEM images of the effects of farnesol and F-PLGA nanoparticles on *C. albicans* ultrastructure

development in various conditions and its inhibitory effect on some pathogenic microorganisms, its use for therapeutic purposes attracts great attention (Costa et al. 2019). In the literature, it is stated that farnesol destroys the cell membranes of bacteria and thus increases the proton permeability by affecting cell-membrane functions. Farnesol is also an interesting component in terms

of antibiofilm strategies. In a study by Sebaa et al., specific antibiofilm effects of farnesol and tyrosol, which are quorum-sensing molecules, were demonstrated on *Candida* isolates. However, this effect was independent of the fungistatic or fungicidal effect. The researchers stated that by the addition of exogenous quorum-sensing molecules, the related mechanism may be

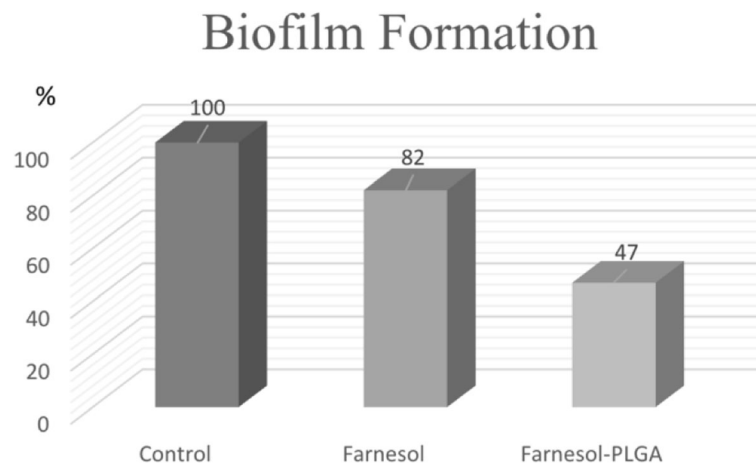


Fig. 6 Demonstration of biofilm formation according to XTT test results

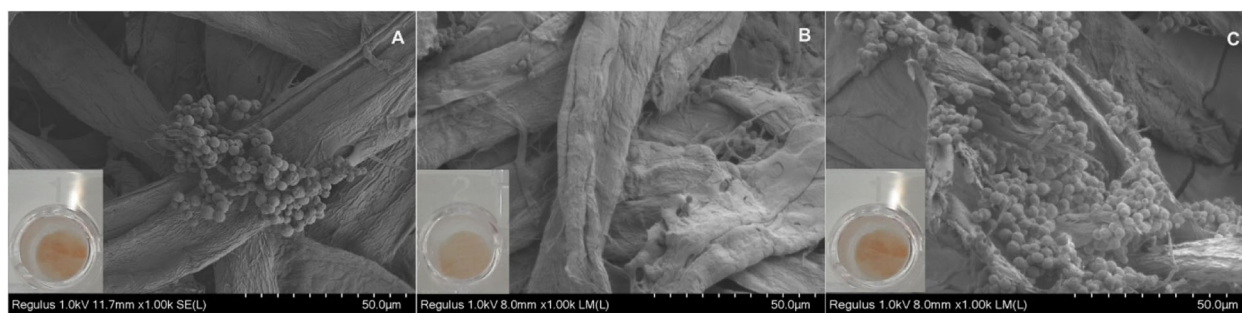


Fig. 7 SEM images of the *C. albicans* cells treated with farnesol alone and F-PLGA NPs on biofilm formation

disrupted or the adhesion of microorganisms on the surface may be limited (Sebaa et al. 2019). On the other hand, farnesol, a hydrophobic drug, has been reported to have antimicrobial effects against certain pathogens such as *S. mutans*. However, due to the limits of solubility associated with hydrophobicity and poor biofilm penetration, the use of high concentrations of drug is required in biofilm treatment. Studies have shown that when polymeric nanoparticles are used to increase the efficacy of farnesol, its effectiveness on oral biofilm increases (Sims et al. 2019). On the other hand, the interactions between biofilm and the active substance used are the most important factors in determining the effectiveness of the treatment. The EPS structure in the biofilm is heterogeneous and limits the penetration of antimicrobials (Wang et al., 2016). Electrostatic, hydrophobic, and other non-covalent interactions of active substances that interact with both microorganism and EPS structure are important in antibiotic film strategies.

Ability of farnesol to prevent yeast-hyphae transmission is highly promising both in the control of infection and in optimizing the antifungal effect of other drugs (Katragkou et al, 2014). Arasoğlu et al. investigated the antimicrobial effects of quercetin-loaded PLGA nanoparticles on four different bacterial isolates. In the study, the efficacy of bacterial isolates showed significant differences, and authors have reported that this may be due to different bacterial cell wall structures. Both quercetin and quercetin-laden NPs have been reported to be effective only on gram-positive bacteria, and the complex cell wall structure in gram-negatives constitutes a barrier for bacteria (Arasoglu et al., 2017). Costa et al. (2019) investigated the effects of farnesol and miconazole co-encapsulated in biodegradable and biocompatible chitosan nanoparticles on the vulvovaginal candidiasis murine model. The authors reported that chitosan nanoparticles containing farnesol and miconazole are effective in inhibiting fungal growth. In addition, it was reported that farnesol-containing chitosan nanoparticles can reduce the pathogenicity of infection due to lack of inflammation (Costa et al. 2019). Esfandyari-Manesh et al.

reported that it is very advantageous to use nanoparticles to arrest hydrophobic compounds with antimicrobial properties. These advantages include increased hydrophilicity, sustained release, and better penetration characteristics (Esfandyari-Manesh et al. 2013).

In our study, F-PLGA NPs were synthesized, characterized, and investigated for antimicrobial and antimicrobial properties on *C. albicans*. The synthesized F-PLGA NPs were approximately 140 nm in size, with a PDI of 0.463 and a positive zeta potential (658.5). Since the samples were prepared in aqueous solution in the zeta potential test, the nanoparticles were observed in larger size, whereas the dried samples were examined in TEM, so that the sample sizes were smaller. Roger et al. reported that nanoparticles with positive zeta potential between 50 and 300 nm are more effective especially for mucosal applications (Roger et al., 2010). In our study, NP dimensions were found to be in this range and zeta potential was positive and quite high. According to our ^1H NMR data, peaks of farnesol molecule were contained with peaks of PVA molecule in the 1–2 ppm range. On the other hand, all of the proton peaks of farnesol molecule except for OH group proton were observed in the spectrum of farnesol-loaded PLGA nanoparticles. It was concluded that in particular, the PLGA nanoparticle has chemically interacted with the OH functional group of the farnesol molecule.

In the present study, when ultrastructural effects of farnesol on planktonic *Candida* cells were examined by TEM, normal round-oval morphology was preserved in most of the cells, but few cells had wall and membrane damage and cytoplasm density in electrons. In the cells treated with F-PLGA NPs, the damaged findings were more increased, the number and volume of vacuoles increased, and deterioration in cell morphology and cell wall membrane expansion were observed. Decanis et al. investigated the effects of exogenous farnesol at different concentrations (10, 100, 300 μM) on a *C. albicans* strain that does not produce endogenous farnesol. It has been shown that farnesol causes changes in cell wall shape in

TEM findings. Also, a visible disconnection was detected between the cell wall and the cytoplasm. In addition, the vacuoles were observed in the cytoplasm (Décánis et al. 2011). Similar findings were found in also our study, but the ultrastructural damaging effects of F-PLGA NPs on the cell were found to be greater than farnesol alone. On the other hand, SEM micrographs obtained as a result of treatment of farnesol at different concentrations indicate deterioration in the external morphology of the *Candida* cells. The researchers stated that this situation indicates a decrease in cytosolic volume (Décánis et al. 2011).

In our study, farnesol and F-PLGA NPs were applied to *Candida* cells before biofilm formation and prebiofilm activities were investigated in both XTT and SEM analyses. Both analyses demonstrated the suppressive and reducing effects of F-PLGA NPs on biofilm formation, which were more potent than farnesol. In the literature, it is reported that farnesol prevents hyphal formation and biofilm development. On the contrary, tyrosol, another quorum-sensing molecule, stimulates hyphal formation. In the study of Alem et al., farnesol was added at three different concentrations (50 μ M, 100 μ M, and 1 mM) and at different stages of the formation of *C. albicans* biofilm. Researchers have reported that early stages are sensitive to farnesol and these findings support our results (Alem et al. 2006). Katragkou et al. investigated the effects of combined use of farnesol with micafungin, fluconazole, and amphotericin B on *C. albicans* biofilms. Farnesol showed a synergistic or additive effect with these antifungals and structural changes were observed in the biofilm. The maximum combined effect was dependent on farnesol concentration (Katragkou et al., 2015). Chen et al. reported that the resistance of *C. albicans* biofilms to antifungals is associated with the enzymes CYR1 and PDE2, which are responsible for the synthesis and degradation of the cyclic AMP signaling pathway, and that farnesol reduces the antifungal resistance of *C. albicans* biofilms (Chen et al. 2018).

Conclusion

As a result, farnesol loaded to PLGA nanoparticles successfully by emulsion evaporation method. Our results demonstrate promising potential inhibitory effects of F-PLGA nanoparticles when applied to both planktonic *Candida* cells and biofilm formation. Especially in F-PLGA, a much smaller amount (22.5% of the total volume) of active substance was used compared to the use of farnesol alone and a similar effect to the efficacy of farnesol was observed. Inhibiting effects of hydrophobic molecule farnesol on mature *Candida* biofilms have also been reported, but nanoencapsulation systems can increase the effectiveness and potential of this interesting component in terms of their small size and large penetration areas. With detailed studies, it will be possible to

obtain data that can shed light on the use of this molecule which regulates an important virulence factor such as yeast-hyphae transition. Further research is needed on the synergistic applications of the antifungals available with farnesol or on their effects when used with different biocompatible carriers.

Abbreviations

PLGA: Poly(DL-lactide-co-glycolide); F-PLGA Nps: Farnesol-loaded poly(DL-lactide-co-glycolide) nanoparticles; XTT: (Sodium 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate); PBS: Phosphate-buffered saline; CYR1: The adenylate cyclase enzyme polypeptide; PDE2: Phosphodiesterase 2; AMP: Adenosine monophosphate

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Author's contributions

The author read and approved the final manuscript.

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Competing interests

The author declares no conflict of interest.

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