Infection, Genetics and Evolution 70 (2019) 36–41

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research Paper

The effect of released new synthetic peptide from nanofibrous scaffold of peptide/Poly (Vinyl Alcohol)/Poly L-Lactic Acid on expression of Secretory aspartyl proteinases 4 to 6 genes of *Candida albicans*

Nasser Keikha[a,1], Mohammad Hossein Yadegari[a,*], Masoumeh Rajabibazl[b], Jafar Amani[c], Symzar Hosseinzadeh[d,e]

[a] Department of Medical Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Clinical Biochemistry, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Applied Microbioloy Research Center, System Biology and Poisonings Institute, Baqiatallah University of Medical Sciences, Tehran, Iran
[d] Department of Tissue engineering and Applied Cell Sciences, School Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Stem Cell Technology Research Center, Tehran, Iran

ARTICLE INFO

Keywords:
Nanofibers
Candida albicans
Gene expression
Antifungal

ABSTRACT

Secreted aspartyl proteinases (Saps), are gene products that have been shown to directly contribute to *Candida albicans* pathogenicity. Despite the clear difficulties of systemic *C. albicans* infections control, Antimicrobial peptides (AMPs) are regarded as one of the most promising alternatives in this regard. Recently, drug-loaded electrospun nanofibers have attracted a great deal of attention. In this study we have established the nanofibrous scaffold of new synthetic peptide/Poly (Vinyl Alcohol)/Poly L-Lactic Acid on expression of Secretory aspartyl proteinases 4 to 6 genes of *C. albicans* in comparison with free peptide. We designed new synthetic Antimicrobial peptide (AMPs) used bioinformatics tools to predict structure and stabilities. The electrospinning method was used to produce the polymeric nanofibers. Scanning Electron Microscopy (SEM) was used to measure the nanofibers diameters and morphology. To analyze the expression of SAP4, SAP5 and SAP6 genes, RNA was extracted from clinical isolates of *C. albicans* before and after treatment with subinhibitory concentrations of new synthetic peptide on nanofibrous scaffold of new synthetic peptide/PVA/PLLA and then the cDNA was synthesized and used for Real-time PCR assay. Scanning electron microscopy (SEM) images showed that the morphology, the diameter, are affected from peptide. Reletive quantitative Real-time PCR results revealed that the mRNA levels of SAP4, SAP5 and SAP6 genes significantly decreased after treatment with nanofibrous scaffold of new synthetic peptide/PVA/PLLA ($P < .05$). Electrospun nanofibrous of new Synthetic Peptide/PVA/PLLA is effective in downregulating of expression SAP4, SAP5 and SAP6 genes of *C. albicans* strains.

1. Introduction

Candida albicans is the most pathogenic fungus among members of the genus Candida. This species is equipped with several virulence factors including genes coding adhesion molecules, metabolic flexibility, resistance to stressful conditions, yeast-to-hyphal transition and production of hydrolytic and proteolytic enzymes (Calderone and Fonzi, 2001). The extra cellular hydrolytic enzyme, especially the secretory aspartyl proteinases (Saps), are one a few gene products that have been shown to directly contribute to *C. albicans* pathogenicity (Mardegan et al., 2006). *C. albicans* is able to produce a family of 10-member secretory aspartyl proteinases among which Sap4, Sap5 and Sap6 are expressed upon hyphal formation. Degradation of tissue barriers during invasion and destruction of host defense molecules are other responsibilities of aspartic proteases in *C. albicans* cells (Lermann and Morschhauser, 2008; Naglik et al., 2004). Invasive candidiasis is known as the major cause of mortality and morbidity in lateonset infection in premature infants (Ericson et al., 2016) and immunocompromised patients as a result of AIDS, cancer chemotherapies or organ transplantation. Despite the advances in treatment of fungal infections, there are several reports about drugs resistant fungal strains, treatment failure and the scarcity of antifungal agents with low toxicity

* Corresponding author at: Department of Medical Mycology, Faculty of Medical Sciences, Tarbiat Modares University, P.O.Box: 14117-13116, Tehran, Iran.
E-mail address: yadegarm@modares.ac.ir (M.H. Yadegari).
[1] Medical Laboratory Sciences Department, Infectious Diseases and Tropical Medicine Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran.

https://doi.org/10.1016/j.meegid.2019.02.015
Received 15 December 2017; Received in revised form 29 November 2018; Accepted 16 February 2019
Available online 18 February 2019
1567-1348/

for systemic *C. albicans* infections (Formosa et al., 2013; Gow and Hube, 2012). Antimicrobial peptides (AMPs) are regarded as one of the most promising alternatives in this regard (Yeung et al., 2011). AMPs have recently become the focus of considerable interest as a candidate for a new type of antibiotic, due primarily to their potency against pathogenic microbes that are resistant to conventional antibiotics, as well as their broad-spectrum activity (Bulet et al., 2004). They act as the first line of defense and encompass direct antimicrobial activity against a broad spectrum of invading pathogens including bacteria, fungi, viruses and protozoa by using different modes of action as described previously (Jenssen et al., 2006; Reddy et al., 2004). Thus, AMPs can be potentially promising candidates for development of novel therapeutic agents against pathogens. Recently, drug-loaded electrospun nanofibers have attracted a great deal of attention, because they offer higher drug encapsulation efficiency and better structural stability than other drug carriers (Chew et al., 2006; Han et al., 2007). Polymeric drug delivery systems are able to improve therapeutic efficacy, reduce toxicity, and enhance compliance of the patients by delivering drugs at a controlled rate over a period of time to the site of action (Felice et al., 2014). Electrospinning is the method for preparing drug-loaded nanofibers with ultrafine structure, a large surface area to volume ratio, and a high porosity with a small pore size. Among the other nanofiber production methods, electrospinning is the most cost effective one with simple tooling and, it is applicable to produce ultrafine fibers with a simple step-up production for drug delivery applications (İmren et al., 2016). Electrospun nanofibers have been developed for antimicrobial and antifungal drugs, skin tissue scaffolds, wound dressings as well as drug delivery applications includingAlzheimer drugs, cosmeceuticals and genes (Arthanari et al., 2014; Gencturk et al., 2016). Therefore, In the present work we have established the nanofibrous scaffold of new synthetic peptide/Poly (Vinyl Alcohol) (PVA)/Poly L-Lactic Acid (PLLA) on expression of Secretory aspartyl proteinases 4 to 6 genes of *C. albicans* in comparison with free peptide.

2. Matherials and methods

2.1. Peptide design and synthesis

The physico-chemical parameters of the chimeric protein including molecular weight, extinction coefficient, half-life, instability index, theoretical isoelectric point (pI), grand average of hydropathy (GRAVY) and total number of positive and negative residues were obtained using the Expasy ProtParam (http://web.expasy.org/protparam/) (Wilkins et al., 1999). Peptide was synthesize to 85% purity by ShineGene Molecular Biotech,Inc. (Shanghai, China. http://www.synthesisgene.com/peptidesynthesis.html). The characterization and purity estimation of the peptide was performed by using electrospray mass spectrometry (ESMS) and high performance liquid chromatography (HPLC). Peptide was dissolved in sterilized 50% (*v*/v) DMSO/water mixture and subsequently add water/buffer until the desired concentration is achieved and stored at −20 °C.

2.2. Cytotoxicity assay

Human breast cancer MCF-7 cell lines were treated with the novel synthetic peptide for 48 h to evaluate their cytotoxicity related to untreated samples by MTT assays. MCF-7 cells of breast cancer were cultured in 10% Fetal Bovine Serum (FBS) (Rockville, MD, USA) and 100 g/ml Streptomycin, 100 u/ml Penicillin (Sigma). Then, they were maintained at 37 °C in a humidified atmosphere with 5% CO2 until reaching 80% of confleuncy. The MCF-7 cells (5×10^3 cells/well) were seeded onto 96-well plates and after 24 h, different concentrations (*i.e.* ranging from 50 to 400 μg/ml) of the novel synthetic peptide were added to each well. After the 48 h, in each well was added 20 μl (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium) bromide (MTT). The treated cells were then incubated for 3.5 h at 37 °C and 5% CO2. Eventually, after removing of supernatant, 200 μl DMSO were added to the plate and the medium absorbance of the formed formazan was read by spectrophotometer (Elisa reader, Biochrom Anthos 2020 microplate reader) at a wavelength of 570 nm and cell viability was calculated according to the standard protocol.

2.3. Antifungal succeptibility testing

Antifungal activity of the designed peptide was determined according to the standardized broth microdilution method (Clinical and Laboratory Standards Institute (CLSI) document M27-A4). using RPMI 1640 medium without phenol red, an inoculum of 1.5×10^3 cells/ml, and incubation at 37 °C. MICs were evaluted visually after 24 h incubation as the lowest concentration of drug that caused a significant diminution of growth below growth control levels (Pfaller et al., 2004; Ryan et al., 2013). The concentration of Peptide ranged from 0.15 μg/ml to 80.00 μg/ml and all strains of *C. albicans* were tested in triplicate.

2.4. Electrospinning and peptide release time

The electrospinning method was used to produce the polymeric nanofibers. The polymer utilized Poly (L-D-lactic acid) was obtained (Sigma-Aldrich). Chloroform and dimethyl formamide (Sigma-Aldrich) were used to dissolve the PLA. The polymer solution of 7% (*w/v*) was prepared with 30% solvent *N*, *N*-dimethylformamide (DMF) and 70% Chloroform. The mixture was left under continuous agitation with magnetic stirrer at ambient temperature of 24 °C for at least 2 h. 2 mg of novel synthetic peptide was solubilized in 0.25 ml of deionized water. During the electrospinning process, parameters such as voltage 17 kV and distance between the needle and the collector were kept as 16 cm that was the same for PVA, PLLA and peptide–PVA fiber. Earlier, 10% PVA was prepared and then, peptide was solubilized in deionized water under stirring and then was added to the PVA solution. PVA, a synthetic polymer, has been widely used to produce electrospun fiber mats (Shabani et al., 2009). The produced scaffolds were collected on aluminum foils.

Peptide release time from nanofibrous scaffold of new synthetic peptide/PVA/PLLA was assessed by either measuring absorbance at 280 nm. A solution of peptide (at 100 nM) in 1 ml of phosphate-buffered saline, pH 7.4 (PBS) for 48 h (Lozano et al., 2010).

2.4.1. Scanning electron microscopy (SEM)

For morphological analysis of the nanofibers by scanning electron microscopy (Hitachi - SU3500 - Scanning Electron Microscope by Hitachi High Technologies America, Inc.) was used. All of the samples were coated with an ultra-thin gold layer (20 nm) using the Sputter Coat Emitech K550. SEM images were analyzed and captured, and the diameters of the fibers in the scaffold were determined using 5000× magnification by the Image J Tool for Windows version 3.0.

2.5. RNA extraction and quantitative real-time PCR assay

The expression of SAP4, SAP5 and SAP6 genes was measured by quantitative Real-time PCR. Total RNA was extracted by RNA kit (CinnaClon, Karaj, Iran) according to the manufacturer's instructions. RNA extraction of *C. albicans* strains at three times (24, 48 and 72 h) before and after treated with 0.31 μg/ml of the peptide/PVA/PLLA nanofiber and free peptide was done. RNA concentrations and purity were determined spectrophotometrically (eppendorf biophotometer, Germany). Equal amount of RNA (1 μg) were subjected to cDNA synthesis by use of PrimeScript RT reagent Kit (Thermo Scientific). To determine gene expression, the following pairs of primers were used: 5'-AGATATTGAGCCCACAGAAATTCC-3′ and 5'-CAATTTAACTGCAACAG GTCCTCTT-3′ for SAP4; *5'-CAGAATTTCCCGTCGATGAGA-3′* and *5'-CATTGTGCAAAGTAACTGCAACAG-3′* for SAP5;*5'-TTACGCAAAAGGT AACTTGTATCAAGA-3'*and*5'-CCTTTATGAGCACTAGTAGACCAA*

ACG-3′ for SAP6; *5′-CCAGCTTTCTACGTTTCC-3′* and *5′-CTGTAACCAC GTTCAGAC-3′* for The β-actin gene as endogenous reference gene (ACT1). Real-time PCR was performed with a StepOnePlus Real-time PCR system (Applied Bio-Rad, USA) All PCR reaction mixtures contained the following: 12.5 µl qPCR SYBR Green Master Mix high ROX (Ampliqon, Denmark), 1 µl of first strand cDNA, 0.1 Pmol of each primers and dH2O up to the final volume of 25 µl. The program for amplification was 95 °C for 60 s as initial denaturation step followed by the 40-cycled PCR step consisting of 95 °C for 10 s and 72 °C for 30 s. Negative control (water as template) was included in each run. Expression of each investigated gene was normalized to the housekeeping gene ACT1 and analyzed by applying REST (Relative Expression Software Tools) software (2008 V2.0.7). The software uses the comparative Ct method (ΔΔCt) to analyze data. Expression of SAP4, SAP5 and SAP5 genes from cells grown under nanofibrous scaffold of Peptide/PVA/PLLA-treatment condition was indicated as relative expression to that of gene from untreated yeast cells. Each experimental condition was performed in triplicate.

2.6. Statistical analysis

Results are expressed as mean ± SEM. Statistical evaluation was carried out with nonparametric analysis of variance (Kruskal-Wallis). A value of $p < .05$ was considered significant.

3. Results

3.1. The physico-chemical parameters

The average molecular weight of chimeric protein with sequence NALLHHGLNCAKGVLWLNALLHHGLNCAKG was calculated 31.88 kDa. Isoelectric point (pI) was defined as the pH at which the surface of protein is covered with charge but net charge of the protein is 2.3. Acidity of the protein was indicated by pI value (pI < 8.91). Extinction coefficient of chimeric protein at 280 nm was 56.250 M/cm. The biocomputed half-life was > 10 h. Expasy ProtParam classifies the chimeric protein as stable protein with instability index, 7.18. Aliphatic index and GRAVY of chimeric protein was 127.00 and 0.323, respectively.

3.2. Peptide nanofiber release

The release of the peptide/PVA/PLLA spun fibers was higher during the first 18 h, The peptide release from the peptide/PVA/PLLA nanofiber is shown in Fig. 1.

3.3. Cytotoxic evalution

The cytotoxic activity of novel synthetic peptide against MCF-7 cells was measured. As shown in Fig. 2, novel synthetic peptide did not show significant toxicity against MCF-7 cells at concentrations of up to 400 µg/ml. However, mean cell viability at a concentration of 50 µg/ml and 400 µg/ ml was equivalent to 81% and 72%, respectively.

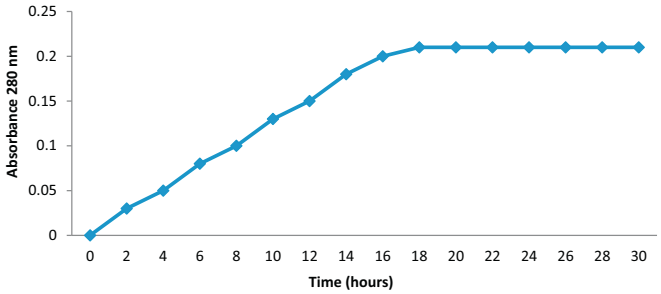

Fig. 1. peptide/PVA/PLLA release analyses from nanofibrous scaffold.

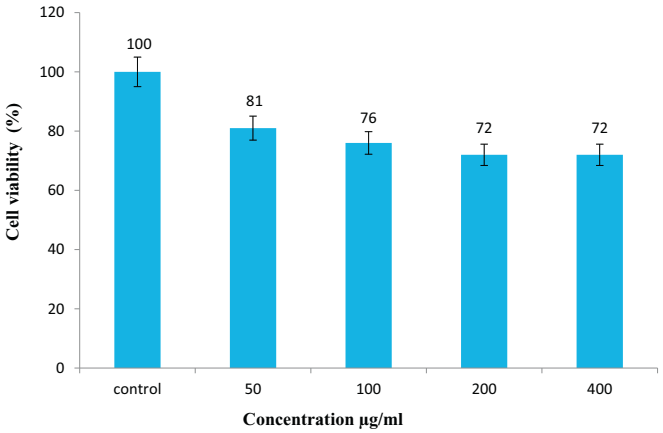

Fig. 2. Cytotoxic effects of different concentrations of novel synthetic peptide against MCF-7 cells of breast cancer.

3.4. Antifungal activity of novel synthetic peptide

The Minimum inhibitory concentrations (MICs) of the novel synthetic peptide and caspofungin (as control antifungal drug) against *C. albicans* strains were determined (Table 1). Evaluation of the MICs values showed that novel synthetic peptide was active against all the *C. albicans* strains. Novel synthetic peptide MICs values ranged from 0.07 µg/ml to 2.5 µg/ml against the *C. albicans* strains. The MICs values of caspofungin ranged from 0.48 µg/ml to 1.95 µg/ml against *C. albicans* isolates.

3.5. Microscopic characterization of nanofibers

To more evaluate the resulting nano-fibers, SEM was performed (Fig. 2a and b). The average diameter of the fibers containing PVA/PLLA without peptide were 400 nm, whereas the average diameter of the fibers containing PVA/PLLA in presence of peptide was 450 nm. The nanofiber morphological characterizations in Fig. 2a and b show that the fiber diameter increases with loading peptide in system. Furthermore, by comparing nanofibers Fig. 2a and b, it is clear that that the PVA/PLLA control fibers are more homogeneous in comparison to the fibers containing peptide.

3.6. Evalution of peptide/PVA/PLLA nanofiber and free peptide on expression of SAP4 to 6 genes of C. albicans strains

C. albicans isolates were evaluted for expression of SAP4-6 genes in treatment and untreated of 0.31 concentrations of peptide/PVA/PLLA nanofiber and free peptide. *C. albicans* clinical vaginal strains expression of SAP4,5 and 6 genes decreased after treatment with peptide/PVA/PLLA nanofiber and free peptide. Expression of SAP4 significantly decreased after 24, 48 and 72 h treatment with peptide/PVA/PLLA nanofiber ($P < .05$) (Fig. 3 (a)). Expression of SAP6 gene decreased after 24, 48 and 72 h treatment with peptide/PVA/PLLA nanofiber and free peptide about 94% and 90%, 99% and 96%, 99% and 98%, respectively, ($P < .03$) (Fig. 3 (c)). Expression of SAP5 in *C. albicans* isolates decreased about 77%, 99% and 94%, respectively after 24, 48

Table 1
In vitro susceptibility testing of clinical vaginal isolates of *C. albicans* (µg/ml).

Agent	MIC range	MIC50	MIC90
Novel synthetic peptide	0.07–2.5	0.03	1.25
Caspofungin	0.48–1.95	0.24	0.97

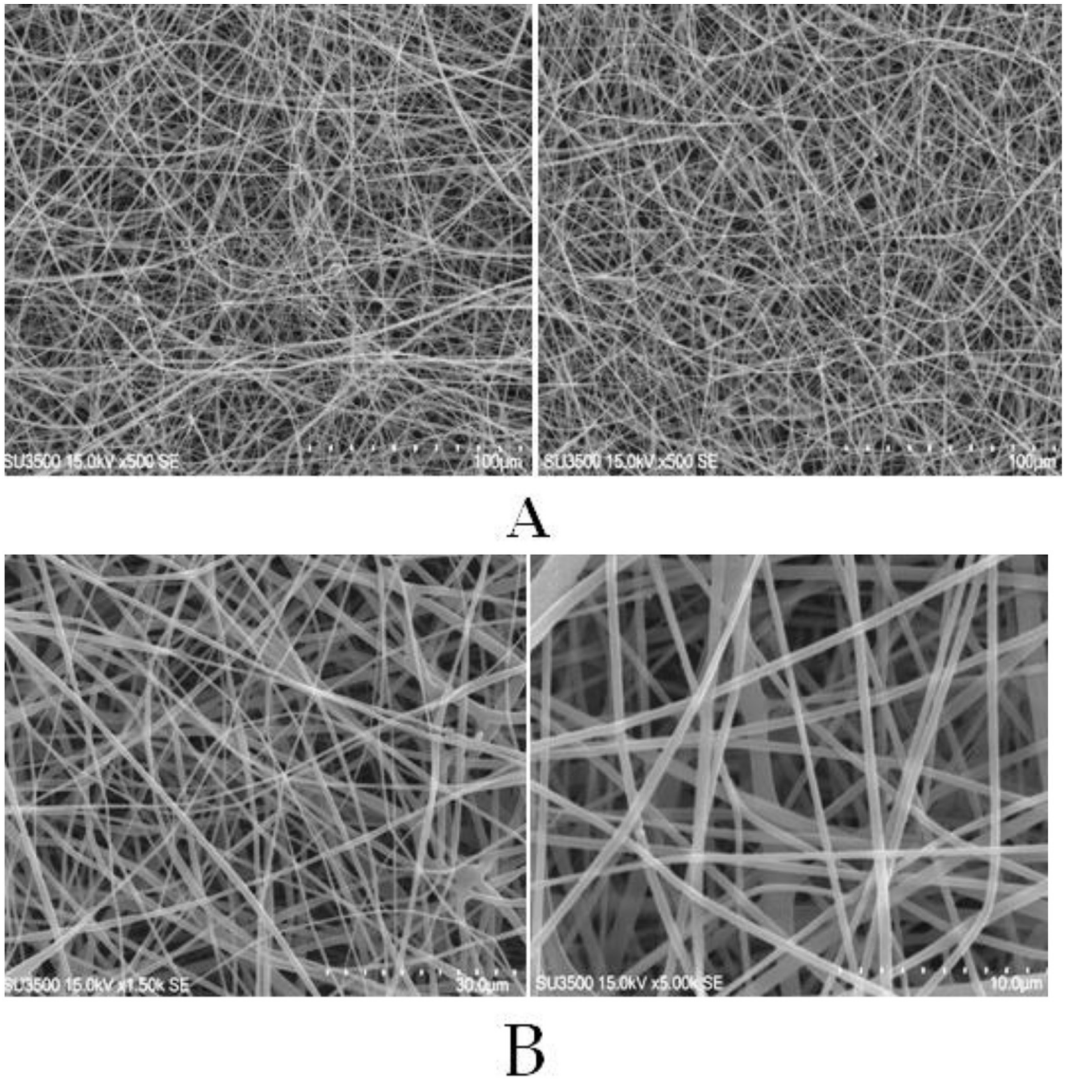

Fig. 3. SEM micrographs: (A) PVA/PLLA fibers without peptide, (B) peptide/PVA/PLLA fibers with 5000× magnification.

and 72 h treatment with peptide/PVA/PLLA nanofiber ($P < .01$) (Fig. 3 (b)).

4. Discussion

In this study we demonstrate the production of nanofibers that loaded new synthetic peptide. At the moment, few antimicrobial peptides have been incorporated into nanofibers (Kumamoto and Vinces, 2005; Yang et al., 2008). According the limited number of suitable and effective antifungal drugs, the continuing increase in the incidence of *Candida* infections, together with increasing drug resistance, highlights the need to discover new and better antifungal agents that target fundamental biological processes and or pathogenic determinants of *C. albicans* (Kumamoto and Vinces, 2005). Therefore, it seems necessary to find new drug agents having the least toxicity, and side effects in the treatment of Candida infections. We attempted to generate new synthetic peptide against *C. albicans* and evalution of released new synthetic peptide from nanofibrous scaffold of peptide/Poly (Vinyl Alcohol) (PVA)/Poly L-Lactic Acid (PLLA) on expression of Secretory aspartyl proteinases 4 to 6 genes of *C. albicans* before and after treatment. Evaluation of MIC values showed that new synthetic peptide was active against *C. albicans* strains, with a MIC values ranging from 0.07 μg/ml to 2.5 μg/ml. As in the case of many other peptides, including K19Hc (Jang et al., 2006), Arenicin-1 (Cana and Dong, 2009), and,KABT-AMP and uperin 3.6 (Lum et al., 2015), all of which exhibited antifungal effect, new synthetic peptide was also anticipated to exhibit antifungal properties. New synthetic peptide–PVA-loaded nanofibrous membranes were fabricated by electrospinning PVA. PVA, a synthetic polymer, has been widely used to produce electrospun fiber scaffold (Peresin et al., 2010) and the polymeric nanofibers become important class of biomaterials (VAZ et al., 2017). Furthermore, PVA nanofibers have been applied in different fields, such as enzyme immobilization, electrode materials, sensors and biomedical applications (Agarwal et al., 2008; Meinel et al., 2012). Secretory aspartyl proteinases (SAPs) from *C. albicans* are encoded by a multi-gene family and are considered to be putative virulence factors for candidiasis. SAP4–6 mRNAs were first detected during hyphae formation and were assumed to play roles in the development of disseminated candidiasis (Chen et al., 2002). Findings of the study showed decreased genes expression of SAP4–6 in *C. albicans* isolates in treatment with nanofibrous scaffold of peptide/PVA/PLLA and free peptide (Fig. 3). Also some researches claimed that the peptide/PVA nanofibers the ability to control fungal infection and their potential applications in the dental field (Viana et al., 2015; Virlan et al., 2016). nanofibrous scaffold of peptide/PVA/PLLA in reducing the expression of SAP5 and SAP6 genes, It was more effective than the free peptide. It seems that mechanism of the peptide/PVA/PLLA was DNA damage.

According to Fig. 3 the best time to apply the peptide/PVA/PLLA

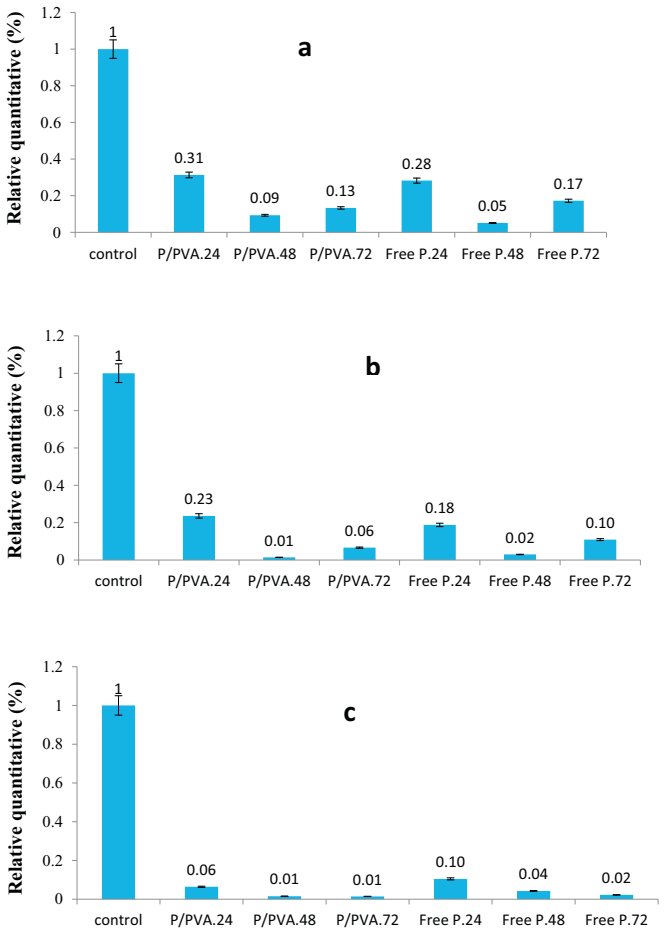

Fig. 4. Expression of SAP4 (a), SAP5 (b) and SAP6 (c) genes after treatment with peptide/PVA/PLLA nanofiber scaffold (P/PVA) and Free Peptide (Free P) compared to pre-treatment. Control: sample before treatment. P/PVA24: 24 h treatment with peptide/PVA/PLLA nanofiber scaffold. P/PVA48: 48 h treatment with peptide/PVA/PLLA nanofiber scaffold. P/PVA72: 72 h treatment with peptide/PVA/PLLA nanofiber scaffold. Free P.24: 24 h treatment with Free peptide. Free P.48: 48 h treatment with Free peptide. Free P.72: 72 h treatment with Free peptide. The results were normalized against ACT1 housekeeping gene expression, which was assigned a value of 1 ($P < .05$).

and free peptide effects on down-regulation of SAP4, SAP5 and SAP6 genes expression was 48 h after treatment. Peptide/PVA/PLLA scaffold and free peptide showed a similar effect in reducing the expression of SAP5 and SAP6 gene 72 h after treatment. Down-regulation of SAP4, SAP5 and SAP6 genes expression were observed in peptide/PVA/PLLA scaffold. Therefore, nanofibrous scaffold of peptide/PVA/PLLA is effective in reduction on expression of SAP4-6 clinical isolates of *C. albicans* (Fig. 4).

5. Conclusion

Nanofibrous scaffold of peptide/PVA/PLLA has reduced the expression of SAP5 and SAP6 genes, It was more effective than the free peptide. Electrospun nanofibrous scaffold of peptide/PVA/PLLA may be useful as down-regulation of SAP4, SAP5 and SAP6 genes expression and drug delivery systems. Also, new synthetic peptide recommended to as preventing pathogenicity of *C. albicans*.

Funding

This work was supported by the Tarbiat Modares University, Tehran, Iran [IR.TMU.REC.1394.82].

Acknowledgments

The authors would like to thank the Tarbiat Modares University for supporting this study.

References

Agarwal, S., Wendorff, J.H., Greiner, A., 2008. Use of electrospinning technique for biomedical applications. Polymer 49, 5603–5621.
Arthanari, S., Mani, G., Jang, J.H., Choi, J.O., Cho, Y.H., Lee, J.H., Cha, S.E., Oh, H.S., Kwon, D.H., Jang, H.T., 2014. Preparation and characterization of gatifloxacin-loaded alginate/ poly (vinyl alcohol) electrospun nanofibers. Cells Nanomed. Biotechnol. 44, 846–852.
Bulet, P., Stocklin, R., Menin, L., 2004. Anti-microbial peptides: from invertebrates to vertebrates. Immunol. Rev. 198, 169–184.
Calderone, R.A., Fonzi, W.A., 2001. Virulence factors of Candida albicans. Trends Microbiol. 9, 327–335.
Cana, P., Dong, G.L., 2009. Fungicidal effect of antimicrobial peptide arenicin-1. Biochim. Biophys. Acta Biomembr. 1788, 1790–1796.
Chen, Y.C., Wu, C.C., Chung, W.L., Lee, F.J., 2002. Differential secretion of Sap4-6 proteins in Candida albicans during hyphae formation. Microbiology 148, 3743–3754.
Chew, S.Y., Wen, Y., Dzenis, Y., Leong, K.W., 2006. The roleof electrospinning inthe emerging field of nanomedicine. Curr. Pharm. Des. 12, 4751–4770.
Ericson, J.E., Kaufman, D.A., Kicklighter, S.D., Bhatia, J., Testoni, D., Gao, J., Smith, P.B., Prather, K.O., Benjamin, D.K., 2016. Fluconazole prophylaxis for the prevention of candidiasis in premature infants: a meta-analysis using patient-level data. Clin. Infect. Dis. 63, 604–610.
Felice, B., Prabhakaran, M.P., Rodríguez, A.P., Ramakrishna, S., 2014. Drug delivery vehicles on a nano-engineering perspective. Mater. Sci. Eng. C Mater. Biol. Appl. 1, 178–195.
Formosa, C., Schiavone, M., Martin-Yken, H., Francois, J.M., Duval, R.E., Dague, E., 2013. Nanoscale effects of Caspofungin against two yeasts species; Saccharomyces cerevisiae and Candida albicans. Antimicrob. Agents Chemother. 57, 3498–3506.
Gencturk, A., Kahraman, E., Güngör, S., Özhan, G., Özsoy, Y., Sarac, A.S., 2016. Polyurethane/hydroxypropyl cellulose electrospun nanofiber mats as potential transdermal drug delivery system: characterization studies and in vitro assays. Artif. Cells Nanomed. Biotechnol. 45, 655–664.
Gow, N.A., Hube, B., 2012. Importance of the Candida albicans cell wall during commensalism and infection. Curr. Opin. Microbiol. 15, 406–412.
Han, X.J., Huang, Z.M., He, C.L., Liu, L., 2007. Preparation and characterization of core-shell structured nanofibers by coaxial electrospinning. High Perform. Polym. 19, 147–159.
İmren, E., Sedef Erdal, M., Sevgi, G., 2016. Electrospinning method to produce drug-loaded nanofibers for topical/ transdermal drug delivery applications. J. Fac. Pharm. Istanbul 46, 49–69.
Jang, W.S., Kim, H.K., Lee, K.Y., Kim, S.A., Han, Y.S., Lee, I.H., 2006. Antifungal activity of synthetic peptide derived from halocidin, antimicrobial peptide from the tunicate, Halocynthia aurantium. FEBS Lett. 580, 1490–1496.
Jenssen, H., Hamill, P., Hancock, R.E.W., 2006. Peptide antimicrobial agents. Clin. Microbiol. Rev. 19, 491–511.
Kumamoto, C.A., Vinces, M.D., 2005. Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence. Cell. Microbiol. 7, 1546–1554.
Lermann, U., Morschhauser, J., 2008. Secreted aspartic proteases are not required for invasion of reconstituted human epithelia by Candida albicans. Microbiology 15, 3281–3295.
Lozano, D., Manzano, M., Doadrio, J.C., Salinas, A.J., Vallet-Regí, M., Gómez-Barrena, E., Esbrit, P., 2010. Osteostatin-loaded bioceramics stimulate osteoblastic growth and differentiation. Acta Biomater. 6, 797–803.
Lum, K.Y., Tay, S.T., Le, C.F., Lee, V.S., Sabri, N.H., Velayuthan, R.D., Hassan, H., Sekaran, S.D., 2015. Activity of novel synthetic peptides against Candida albicans. Sci. Rep. 12, 9657.
Mardegan, R.C., Foglio, M.A., Gonçalves, R.B., Hofling, J.F., 2006. Candida albicans proteinases. Braz. J. Oral Sci. 5.
Meinel, A.J., Germershaus, O., Luhmann, T., Merkle, H.P., Meinel, L., 2012. Electrospun matrices for localized drug delivery: current technologies and selected biomedical applications. Eur. J. Pharm. Biopharm. 81, 1–13.
Naglik, J., Albrecht, A., Bader, 2004. *Candida albicans* proteinases and host/pathogen interactions. Cell Microbiol. 6, 915–926.
Peresin, M.S., Habibi, Y., Vesterinen, A.H., Rojas, O.J., Pawlak, J.J., Seppälä, J.V., 2010. Effect of moisture on electrospun nanofiber composites of poly(vinyl alcohol) and cellulose nanocrystals. Biomacromolecules 13, 2471–2477.
Pfaller, M.A., Messer, S.A., Boyken, L., Rice, C., Tendolkar, S., Hollis, R.J., Diekema, D.J., 2004. Further standardization of broth microdilution methodology for in vitro susceptibility testing of caspofungin against Candida species by use of an international collection of more than 3,000 clinical isolates. J. Clin. Microbiol. 42, 3117–3119.
Reddy, K.V.R., Yedery, R.D., Aranha, C., 2004. Antimicrobial peptides: premisesand promises. Int. J. Antimicrob. Agents 24, 536–547.
Ryan, K., Shields, M., Hong, N., Ellen, G., Press, C.L.U., Cornelius, J.C., 2013. Anidulafungin and Micafungin MIC breakpoints are superior to that of caspofungin for identifying FKS mutant Candida glabrata strains and echinocandin resistance. Antimicrob. Agents Chemother. 57, 6361–6365.
Shabani, I., Haddadi-Asl, V., Seyedjafari, E., Babaeijandaghi, F., Soleimani, M., 2009. Improved infiltration of stem cells on electrospun nanofibers. Biochem. Biophys. Res. Commun. 382, 129–133.

VAZ, B.S., COSTA, J.A.V., MORAIS, M.G., 2017. Production of polymeric nanofibers with different conditions of the electrospinning process. Matéria (Rio de Janeiro) 22.
Viana, J.F., Carrijo, J., Freitas, C.G., Paul, A., Alcaraz, J., Lacorte, C.C., Migliolo, L., Andrade, C.A., Falcão, R., Santos, N.C., Gonçalves, S., Otero-González, A.J., Khademhosseini, A., C, D.S., L, F.O., 2015. Antifungal nanofibers made by controlled release of sea animal derived peptide. Nanoscale 7, 6238–6246.
Virlan, M.J., Miricescu, D., Radulescu, R., Sabliov, C.M., Totan, A., Calenic, B., Greabu, M., 2016. Organic nanomaterials and their applications in the treatment of oral diseases. Molecules 21.
Wilkins, M.R., Gasteiger, E., Bairoch, A., Sanchez, J.C., Williams, K.L., Appel, R.D., Hochstrasser, D.F., 1999. Protein identification and analysis tools in the ExPASy server. Methods Mol. Biol. 112, 531–552.
Yang, E., Qin, X., Wang, S., 2008. Electrospun crosslinked polyvinyl alcohol membrane. Mater. Lett. 62, 3555–3557.
Yeung, A.T., Gellatly, S.L., Hancock, R., 2011. Multifunctional cationic host defence peptides and their clinical applications. Cell. Mol. Life Sci. 68, 2161–2176.