Original Article

The effect of nanochitosans particles on *Candida* biofilm formation

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Abstract

Background and Purpose: In people wearing dentures, the growth of various *Candida* species under the prosthesis leads to the formation of biofilm, which can play the role of a reservoir for *Candida* and other kinds of microbes. Since nano-chitosan particles can cause lasting antimicrobial activity, a more recent approach that utilizes acrylic resins with nano-chitosan particles is proposed. Therefore, we aimed to study the inhibitory effect of nano-chitosan particles on the biofilm formation of *Candida* species in acrylic resins.

Materials and Methods: In this analytical in-vitro study, acrylic resins with nano-chitosan particles with concentrations of 0, 1%, 5%, and 10% were put adjacent to the suspension of *Candida* cells isolated from the individuals' mouth and biofilm formation on resins was measured and compared. Finally, the data were analyzed using Kruskal-Wallis and Chi-square tests.

Results: The observed differences between unmodified acrylic resin (control) and acrylic resin with nano-chitosan particles in terms of biofilm formation were significant ($P<0.05$), but no significant difference was found in the formation of biofilm species on resins.

Conclusion: Biofilm formation of *Candida* species depends on acrylic resin type, in a way that by adding nano-chitosan particles to acrylic resins, biofilm formation of *Candida* species was significantly reduced. To decrease the organization of biofilm and denture stomatitis, the use of acrylics with nano-chitosan particles in producing dentures is recommended.

Keywords: Acrylic resins, Biofilm, *Candida* species, Denture, Nano-chitosan Particles

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Introduction

Oral candidiasis is a common opportunistic infection of the oral cavity caused by overgrowth of *Candida* species, particularly *Candida albicans*. The prevalence of this infection varies according to type and other ripening agents [1]. Incidence of this infection is more frequent among the elderly, especially those wearing dentures [2]. *Candida* virulence is a major factor associated with the pathogenesis of oral candidiasis, which depends on some other factors such as adherence, durability, dimorphism, germ tube, phenotype, and hydrolase production (i.e., lipases and proteases).

Increased pathogenesis occurs as a result of inefficient host defense system and synergic effect of bacteria [3]. In people wearing full dentures, the part of the prosthesis which is in contact with the tissue is covered with a relatively thick layer of bacteria and fungi; this phenomenon is known as biofilm formation [4]. In the development of denture stomatitis, which is observed in 65% of the prosthesis wearers, biofilm formation is of paramount importance and may play the role of a reservoir for *Candida* and other varieties of germs. Formation of biofilms on the mucosal surfaces of the prosthesis depends on several factors, particularly permeability and roughness of the surface [5]. A variety of microorganisms are able to grow in biofilm structure. Biofilms comprise of either single or multiple microbial species. Such organisms are encapsulated in a polysaccharide bed, which are formed on living surfaces like mucosal tissues and non-living surfaces like medical devices [6, 7].

Chitosan is a derivation of chitin and is obtained

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by deacetylation process of chitin [8]. It consists of a β-(1, 4)-linked-D-glucosamine residue with the amine groups randomly acetylated. The amine and –OH groups endow chitosan with many special properties, making it applicable in many areas and easily available for chemical reactions. Chitosan can interact with polyanions to form complexes and gels. Since chitosan is a harmless, non-toxic, non-irritant, biodegradable substance and renewable to nature [9], it is applied in various sectors including biotechnology [10], pharmaceutical and medical industry [11], improved sanitation [12], cosmetics industry [13], and food industry [14].

Micro-particles and nano-chitosan particles are used in tissue engineering, drug delivery, and transfer of DNA vaccine [15]. The feature of chitosan bacteria and fungi removal has been reported for a wide variety of bacterial and fungal strains [15, 16]. Due to its nature of microbe removal and low solubility in water as well as solubility in acid in addition to its high durability in water, it is currently highly regarded; however, there are some restrictions on the use of chitosan. Its microbial enumeration property, non-solution, and high durability in water make it attract much attention [17]. In fact, germicidal effect of chitosan depends on various factors including the fungi species, the medium pH, as well as the concentration, solubility, and molecular weight of chitosan. Today, the ability of nano-particles to inhibit biofilm formation in the oral cavity is of great significance [18].

Although acryl is the main component in replacing the lost tissues in dental patients, not much attention has yet been paid to antimicrobial properties of acrylic resins. Given the fact that nystatin, fluconazolem, and amphotericin B are the current treatments for candidiasis, use of such drugs and drug resistance to this type of topical treatment should be considered [19]. Thus, utilization of anti-fungal material in acryl can play an effective role in preventing biofilm formation on dentures. As the anti-fungal effect of chitosan compounds and its nano-particles is known [14, 16], we aimed to prepare acrylic material containing nano-chitosan particles and study biofilm formation of *Candida* species so as to be able to decrease biofilm formation and denture-induced stomatitis.

Materials and Methods

This in-vitro, analytical study was performed on 100 oral samples taken from people admitted to Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The in-vitro tests were performed through the following steps, and 31 samples were found positive:

1. Preparation of samples

Two sterile swabs rubbed twice to the upper palate were placed in 1 ml sterile saline and were transported to the laboratory.

2. Preparation of fungal samples for testing

First, direct microscopy test was performed on the samples. *Candida* species were detected using Sabouraud dextrose agar with chloramphenicol and CHROMagar Candida and were cultivated and incubated at 30°C for 24 hours. The identity of the species isolates was determined using the API20C-AUX system and considering the ability of the germ tube production.

3. Preparation of a suspension of Candida yeast cells

Using a loop and under sterile conditions, the 24-hour yeast cells were taken from the medium and transferred into tubes with screw caps containing physiological serum. Homogenized by a mixer and using a spectrophotometer at a wavelength of 520 nm and optical density of 0.38, a yeast suspension was prepared with a cell density of 1×10^7 CFU/ml [20].

4. Preparation of nano-chitosan particles

The Chitosan purchased from ACROS Organic (UK) with low molecular weight (1-3 kDa) was prepared as nanoparticles, using water and 1% acetic acid and was mixed vigorously with sodium triphosphate followed by centrifuging for 30 min in the biochemical laboratory of Tarbiat Modarres University, Tehran, Iran. The collected sediment was rinsed several times and then frozen. The powder was ready for use after grinding the frozen substrate.

5. Preparation of modified acryl

To achieve acrylic resins containing nano-chitosan particles, 64 mg nanopowder (containing equal amount of nanoparticles) was blended into 576 mg Transbond XT (3M Unitek, USA) acryl, employing a mixing spatula on a glass slab in a semi-dark setting until a uniform consistency was attained. Afterwards, 200 mg of the 10% w/w blended acryl was mixed with 200 mg of the original composite to obtain 5% w/w containing acryl; similarly, 40 mg of 10% composite was

blended with 360 mg original composite for the 1% w/w acryl.

6. Preparation of acrylic resins

For achieving a smooth surface, after being filled with composite, standard ring-shaped molds with 5 mm diameter were placed between glass slides. Visible light cure (470 nm) was employed for 30 seconds (Bluphase ® 16i, Ivoclar Vivadent AG, Australia). Resins were treated 10 more seconds after utilization of a thin layer of bonding.

7. Adjacent samples with a yeast suspension

First, control and experiment acrylic resins and 1 ml of fungal suspension with a density of 1×10^7 CFU were poured on top of each other into micro-tubes. To minimize test time slips, a negative controller was prepared for each sample series (sterile saline and acrylic discs). The micro-tubes were then placed in the incubator shaker for 90 minutes at 37°C [20]. After washing resins with phosphate buffered saline (PBS), the resins were transferred to new micro-tubes with 1 ml of Saburo broth and were heated in an incubator shaker at 37°C.

8. Investigation of biofilm formation of Candida cells on acryl

After 48 hours, the resins were washed with 2 ml PBS and moved to tubes with 3 ml sterile saline and were shaken using vertex devices with low speed for one minute, so as to separate the yeast cells possibly attached to the samples and suspend them in the saline solution. A volume of 20 ml of the contents of the tube was then cultured on dextrose agar and incubated for 48 h at 37°C. To count the colony forming units (CFUs) responsible for biofilm formation, specimens were sonicated in sterile saline and then vortexed in PBS with 3 mm glass beads. CFU/ml of the microorganism present in the suspension was counted with drop-plate method using serial dilution in microtiter plates. The data was finally analyzed using Kruskal-Wallis, ANOVA, and Chi-square tests.

Results

Of the 31 cases of *Candida* species isolated from the mouths of the subjects, 22 (71%) were male and 9 (29%) were female. The mean age of the subjects was 57.39±6.56 years (age range: 45-69 years). In this study, the distribution frequencies of *Candida* species were 48.4% for 15 cases of *C. albicans,* 35.5% for 11 cases of *C. glabrata,* 9.7% for 3 cases of *C. tropicalis, and* 6.4% for 2 cases of *C. krusei* (Table 1). According to Table 1 and based on the results of one-sample Chi-square test, which represented the distribution of *Candida* species, a P-value of 0.002 was calculated, which indicated a significant difference among the *Candida* species.

As shown in Table 2, the present study showed that the average biofilm formation in *C. albicans* species in concentration of control (acrylic resin without nano-chitosan particles) was 67.14 CFUs and in concentrations of 1%, 5%, and 10% nano-chitosan particles were 51.29, 34.57, and 18.71 CFUs, respectively (*P=0.00*).

The average of biofilm formation in *C. glabrata* species in concentration of control was

Table 1. Distribution frequency of *Candida* species in the subjects' mouth

Candida species	Frequency	Percentage	One-sample Chi-square
C. albicans	15	48.4	X^2=15.32 *P=0.002*
C. glabrata	11	35.5	
C. tropicalis	3	9.7	
C. krusei	2	6.4	
Total	31	100	

One-sample Chi-square test indicated a significant difference among the *Candida* species.

Table 2. Comparison of the *Candida* species biofilm with control acrylic resin and acrylic resin containing nano-chitosan particles

Concentration of nano-chitosan particles *Candida* species	Control	1%	5%	10%	Kruskal-Wallis test
C. albicans	11.23±67.14	10.25±51.29	10.45±34.57	5.96±18.71	*P=0.00*
C. glabrata	9.57±82.17	12.06±60.5	9.85±46.00	8.96±28.50	*P=0.00*
C. krusei	24.04±66.00	23.33±45.50	21.21±41.00	1.41±22.00	*P=0.019*
C. tropicalis	8.88±82.00	9.29±67.67	0.57±51.33	1.15±41.67	*P=0.018*
Kruskal-Wallis	*P=0.11*	*P=0.16*	*P=0.17*	*P=0.19*	

82.17 CFUs and in concentrations of 1%, 5%, and 10% nano-chitosan particles were 60.50, 46.00, and 28.50 CFUs, respectively (*P=0.00*).

The average biofilm formation in *C. krusei* species in concentration of control was 66.00 CFUs and in concentrations of 1%, 5%, and 10% nano-chitosan particles were 66.00, 45.50, 41.00, CFUs respectively (*P=0.019*).

The average biofilm formation in *C. tropicalis* species in concentration of control was 82.00 CFUs and in concentrations of 1%, 5%, and 10% nano-chitosan particles were 67.67, 51.33, and 41.67 CFUs, respectively (*P=0.018*).

Considering the results obtained from the Kruskal-Wallis test and shown in Table 2, the average biofilm formation in different concentrations of nano-chitosan particles in each *Candida* species showed a significant difference, that is, when the concentration of nano-chitosan particles increased, the level of biofilm formation diminished (*P<0.05*).

Table 2 shows that the rate of biofilm formation at concentration of control was highest in the *C. glabrata* species, but lowest in the *C. krusei* species. According to the results of Kruskal-Wallis test, the biofilm formation of the *Candida* species did not show any significant differences for control resins (*P=0.11*).

The rate of biofilm formation on acrylic resins with concentration of 1% nano-chitosan particles was highest in the *C. tropicalis* species, while it was lowest in the *C. krusei* species. According to the Kruskal-Wallis test results, the biofilm formation of *Candida* species on acrylic resin with concentration of 1% nano-chitosan particles demonstrated no significant difference (*P=0.16*).

The rate of biofilm formation on acrylic resins with concentration of 5% nano-chitosan particles was highest in the *C. tropicalis* species, while it was lowest in the *C. albicans* species. According to the results achieved by the Kruskal-Wallis test, no significant difference was found regarding biofilm formation in *Candida* species on acrylic resin with concentration of 5% nano-chitosan particles (*P=0.17*).

The rate of biofilm formation on acrylic resins with concentration of 10% nano-chitosan particles was highest in the *C. tropicalis* species, but the lowest rate was observed in *C. albicans* species. There was no significant difference in terms of biofilm formation in *Candida* species on acrylic resin with concentration of 10% nano-chitosan particles (*P=0.19*).

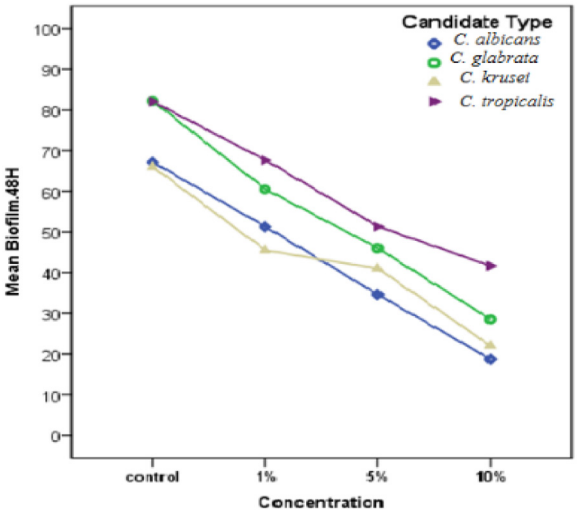

Figure 1. The effect of various concentrations of nano-chitosan particles on the rate of biofilm formation in *Candida* species

Kruskal-Wallis test showed a significant difference in the average of biofilm formation between different concentrations of nano-chitosan particles in each *Candida* species, but did not exhibit any correlation between biofilm formation and *Candida* species.

As shown in Figure 1, when concentration of nano-chitosan particles increases, the rate of biofilm formation reduces.

Discussion

It is currently possible to use new technologies in novel medical and dental instruments [21, 22]. Application of venous catheters, artificial urinary sphincter, artificial joints, as well as prostheses and implants are common in medical sciences [23, 24]. In patients with complete dentures, factors such as the loss of prosthesis harmony with jaws might expose mucus to a large number of fungi clinging to the artificial denture; on the other hand, the negative pressure existing under the upper artificial denture causes saliva antibodies to get away from the area, and as a result, fungi find an opportunity to reproduce between the denture and saliva [25, 26].

Organization of biofilms observed in 65% of the toothless people depends on a number of issues including unevenness, high number of breaches, and roughness of the surface [5]. Since most conventional drugs (nystatin, fluconazole, and amphotericin B in more severe cases) have a transient effect and are not efficient enough, and given that in current treatments for denture stomatitis some issues like drugs' side effects

and pharmaceutical stability should be taken into account, mixing an antimicrobial material into acryl while making the prosthesis may solve some the above-mentioned problems.

Shrestha et al. showed that bacterial biofilm was significantly reduced after exposure to nano-chitosan particles [27]. Chen et al. demonstrated that particle size and incubation time of nano-particles are important factors affecting antimicrobial activity against *Streptococcus mutans* and *Candida albicans* [28].

In their investigation, Silva S et al. showed that mouthwashes containing nano-chitosan particles reduce the biofilm of *Streptococcus mutans* [29]. In another study, Rabea and Sudarshan reported that nano-chitosan cuts down the metabolic activity and durability of *Candida* species biofilms. This phenomenon may be caused by a tension in the biofilm structure that increases the permeability of cell membranes, penetration of nano-chitosan, and fungus removal [30, 31].

Therefore, herein, we purported to incorporate nano-chitosan particles in dental acrylics to study their antimicrobial effects and utilize them for decreasing biofilm formation and denture-induced stomatitis. According to Table 2, the biofilms of *Candida* species were found to depend on acrylic resin type, such that by adding nano-chitosan particles to acrylic resins, the biofilm formation of *Candida* species was significantly reduced.

In a separate investigation, Nikawa and Minake showed that the *Candida*'s capacity of adherence and biofilm formation on denture's textural surface depends on physical (roughness) and chemical properties (material) of acrylic resins [32, 33].

Azcurra exhibited that nano-chitosan particles reduce biofilm formation [34], which is in agreement with our findings, and that the more the concentration of these particles, the less biofilm formation, and thus, there is an inverse relationship between the used nano-particle concentrations and biofilm formation. Mario and Kumar in separate studies reported the dependence of the biofilm formation of the *Candida* species on the catheters [11, 35].

However, in the present study, although *C. glabrata* and *C. tropicalis* had the highest and *C. albicans* and *C. krusei* had the lowest degrees of biofilm formation, respectively, findings of the statistical tests did not show any correlation between biofilm formation and the *Candida* species. Such inconsistency might arise from the difference in isolated resources and separated strains of the species.

Conclusion

Findings of this study showed that nano-chitosan particles have an inhibitory effect on biofilm formation of *Candida* species in the laboratory and it is recommended to use acrylics with nano-particles in producing dentures, so as to decrease the organization of biofilm and denture stomatitis.

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Author's contribution

M.F designed and supervised the study. F.R and A.B provided scientific counseling. M.A and S.S provided technical counseling. Z.S and S.S performed the experiments in the study, and Z.S. drafted the manuscript. The final version of the manuscript was revised by M.F. and Z.**S**

Conflicts of interest

There were no financial interests related to the materials of the manuscript.

Financial disclosure

None.

References

1. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J. 2002; 78(922):455-9.
2. Avendano M, Glymour MM, Banks J, Mackenbach JP. Health disadvantage in US adults aged 50 to 74 years: a comparison of the health of rich and poor Americans with that of Europeans. Am J Public Health. 2009; 99(3):540-8.
3. Hibino K, Samaranayake LP, Hägg U, Wong RW, Lee W. The role of salivary factors in persistent oral carriage of *Candida* in humans. Arch Oral Biol. 2009; 54(7):678-83.
4. Lyon JP, da Costa SC, Totti VM, Munhoz MF, de Resende MA. Predisposing conditions for *Candida* spp. carriage in the oral cavity of denture wearers and individuals with natural teeth. Can J Microbiol. 2006; 52(5):462-7.
5. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. *Candida* -associated denture stomatitis. Aetiology and management: a review: Part1. Factors influencing distribution of *Candida* species in the oral cavity. Aust Dent J. 1998; 43(1):45-50.
6. Weir E, Lawlor A, Whelan A, Regan F. The use of nanoparticles in anti-microbial materials and their characterization. Analyst. 2008; 133(7):835-45.
7. Van Houdt R, Michiels CW. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. Res Microbiol. 2005; 156(5):626-33.
8. Alves NM, Picart C, Mano JF. Self assembling and crosslinking of polyelectrolyte multilayer films of chitosan and alginate studied by QCM and IR

spectroscopy. Macromol Biosci. 2009; 9(8):776-85.
9. Ilium L. Chitosan and its use as a pharmaceutical excipient. Pharm Res. 1998; 15(9):1326-31.
10. Ramnani S, Sabharwal S. Adsorption behavior of Cr (VI) onto radiation crosslinked chitosan and its possible application for the treatment of wastewater containing Cr (VI). React Funct Polym. 2006; 66(9):902-9.
11. Kumar MR, Muzzarelli RA, Muzzarelli C, Sashiwa H, Domb AJ. Chitosan chemistry and pharmaceutical perspectives. Chem Rev. 2004; 104(12):6017-84.
12. Chien PJ, Sheu F, Yang FH. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. J Food Engine. 2007; 78(1):225-9.
13. Yilmaz E. Chitosan: a versatile biomaterial. Adv Exp Med Biol. 2004; 553:59-68.
14. Fujimoto T, Tsuchiya Y, Terao M, Nakamura K, Yamamoto M. Antibacterial effects of Chitosan solution® against Legionella pneumophila, Escherichia coli, and Staphylococcus aureus. Int J Food Microbiol. 2006; 112(2):96-101.
15. Hayashi Y, Ohara N, Ganno T, Ishizaki H, Yanagiguchi K. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. J Dent. 2007; 35(11):871-4.
16. Xu J, Zhao X, Han X, Du Y. Antifungal activity of oligochitosan against Phytophthora capsici and other plant pathogenic fungi in vitro. Pestic Biochem Physiol. 2007; 87(3):220-8.
17. Bae K, Jun EJ, Lee SM, Paik DI, Kim JB. Effect of water-soluble reduced chitosan on Streptococcus mutans, plaque regrowth and biofilm vitality. Clin Oral Investig. 2006; 10(2):102-7.
18. Hernandez-Lauzardo AN, Bautista-Baños S, Velazquez-Del Valle MG, Méndez-Montealvo M, Sánchez-Rivera M, Bello-Perez LA. Antifungal effects of chitosan with different molecular weights on in vitro development of Rhizopus stolonifer (Ehrenb.: Fr.) Vuill. Carbohydr Polym. 2008; 73(4):541-7.
19. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol. 2001; 183(18):5385-94.
20. Jin Y, Samaranayake LP, Samaranayake Y, Yip HK. Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. Arch Oral Biol. 2004; 49(10):789-98.
21. Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clin Microbiol Rev. 2004; 17(2):255-67.
22. Peterson TS. Treatment and prevention of fungal infection. focus on candidemia. New York: Applied Clinical Education; 2007.
23. Ramage G, Saville SP, Thomas DP, Lopez-Ribot JL. *Candida* biofilms: an update. Eukaryot Cell. 2005; 4(4):633-8.
24. Pruthi V, Al-Janabi A, Pereira BJ. Characterization of biofilm formed on intrauterine devices. Indian J Med Microbiol. 2003; 21(3):161-5.
25. Jepson N, Allen F, Moynihan P, Kelly P, Thomason M. Patient satisfaction following restoration of shortened mandibular dental arches in a randomized controlled trial. Int J Prosthodont. 2003; 16(3):409-14.
26. Monsenego P. Presence of microorganisms on the fitting denture complete surface: study 'in vivo'. J Oral Rehabil. 2000; 27(8):708-13.
27. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod. 2010; 36(6):1030-5.
28. Chen CP, Chen CT, Tsai T. Chitosan nanoparticles for antimicrobial photodynamic inactivation: characterization and in vitro investigation. Photochem Photobiol. 2012; 88(3):570-6.
29. Costa EM, Silva S, Madureira AR, Cardelle-Cobas A, Tavaria FK, Pintado MM. A comprehensive study into the impact of a chitosan mouthwash upon oral microorganism's biofilm formation in vitro. Carbohydr Polym. 2014; 101:1081-6.
30. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules. 2003; 4(6):1457-65.
31. Sudarshan NR, Hoover DG, Knorr D. Antibacterial action of chitosan. Food Biotechnol. 1992; 6(3):257-72.
32. Nikawa H, Sadamori S, Hamada T, Satou N, Okuda K. Non-specific adherence of *Candida* species to surface-modified glass. J Med Veterin Mycol. 1989; 27(4):269-71.
33. Miyake Y, Fujita Y, Minagi S, Suginaka H. Surface hydrophobicity and adherence of *Candida* to acrylic surfaces. Microbios. 1986; 46(186):7-14.
34. Azcurra AI, Barembaum SR, Bojanich MA, Calamari SE, Aguilar J, Battellino LJ, et al. Effect of the high molecular weight chitosan and sodium alginate on *Candida albicans* hydrophobicity and adhesion to cells. Med Oral Patol Oral Cir Bucal. 2006; 11(2):E120-5.
35. Tumbarello M, Posteraro B, Trecarichi EM, Fiori B, Rossi M, Porta R, et al. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J Clin Microbiol. 2007; 45(6):1843-50.