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THE EFFECT OF CAFFEINE AND NICOTINE ON METABOLIC ACTIVITY AND BIOFILM ADHESION OF CANDIDA ALBICANS ON 3D PRINTED ACRYLIC RESIN

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Abstract

Purpose: The purpose of this in vitro study was to measure C. albicans' metabolic activity and biofilm adhesion on 3D printed acrylic resin. **Material and methods:** A total of 240 3D printed acrylic resin specimens were constructed. A negative control group with 0 mg/mL of nicotine and 0 mg/mL of caffeine served as the test group for the ten subgroups (n=12), which were assessed for metabolic activity and biofilm development assessment. The remaining 9 subgroups had 8.00 mg/mL of nicotine as well as 9 distinct exponentially ascending caffeine concentrations at the following levels: 0, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00, and 32.00 mg/ml in each segment. Metabolic activity was assessed by using a 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-carboxanilide (XTT). The dislodged biofilm underwent spiral plating at two dilutions (1:100 and 1:1000). 48 hours were spent incubating the plates at 37 C with 5% CO_2. An automated colony-counting device (Protocol 3; Symbiosis) was used to count the yeast colonies and calculate the CFU/ml. **Results:** There was a statistically significant difference ($p<0.05$) between all groups and control group regarding the color changes. While regarding no of colonies (the CFU/ml) there was no significant difference between control group and ((0/8), (0.25/8) and (0.5/8)) while for remaining subgroups there was a significant difference ($p<0.05$). **Conclusion:** The presence of 8 mg/mL of nicotine alone increased the metabolic activity and biofilm formation of C. albicans. In the presence of 8

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mg/mL of nicotine with different caffeine concentrations, overall, caffeine at higher concentrations (1.00, 2.00, 4.00, 8.00, 16.00, and 32.00mg/mL) inhibited biofilm formation of C. albicans on the specimen

Keywords: Caffeine, Nicotine Metabolic Activity, Biofilm Adhesion, Candida Albicans, 3D Printed Acrylic Resin

INTRODUCTION

The denture base is the component that comes into touch with soft tissue. Recent scientific and technological advancements have enabled the use of computer-aided design/computer-aided manufacturing (CAD/CAM) and three-dimensional (3D) printing in the fabrication of denture bases.[1]

Digital technologies offer faster denture manufacture and fewer steps of the labour process, which can lower the likelihood of errors.[2] CAD/CAM technology enables the creation of dentures at a faster rate and with more precision, as well as at a lesser cost than manual production. [3]

Approximately, 45-65% of healthy individuals have Candida albicans in their mouth cavity, by using antifungal medication, the growth of Candida albicans can be slowed down.[4] C. albicans overgrowth can cause changes in the host environment, placing denture users at a greater risk for stomatitis.[5] Caffeine has been regarded as a possible growth inhibitor of fungi [6] , In contrast, it has been discovered that nicotine promotes the growth of Candida albicans and that smokers have a greater prevalence of C. albicans than nonsmokers.[7] Because nicotine alone can enhance the metabolic activity and biofilm development of Candida albicans, smokers who wear detachable prosthesis should be mindful of their nicotine consumption [8]. Caffeine may reduce the metabolic activity and biofilm development of Candida albicans on dentures of individuals who are both smokers and caffeine consumers. [8] Few research have investigated the effect of caffeine and nicotine on C. albicans. Therefore, the authors suggested additional research into the potential of caffeine to reduce C. albicans growth, adhesion, and biofilm formation.

This study aimed to determine if caffeine affects the metabolic activity and biofilm development of C. albicans growing on 3D-printed acrylic resin denture material when nicotine is present, and if so, if this effect varies with caffeine concentration.

MATERIAL AND METHODS

240 3D-printed acrylic resin specimens were created to assess the metabolic activity and biofilm adherence of C. albicans. A negative control group with 0 mg/mL of nicotine and 0 mg/mL of caffeine served as the test group for the ten subgroups (n=12), which were assessed for metabolic activity and biofilm development assessment. The remaining 9 subgroups had 8.00 mg/mL of nicotine as well as 9 distinct exponentially ascending caffeine concentrations at the following levels: 0, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00, and 32.00 mg/ml in each segment.

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The test specimen was designed as an 8×8×2 mm using computer-aided design software (solid works, Dassault Systèmes SolidWorks Corp, France). Specimens were printed vertically with 100 um layer in thickness (z-direction angulated 90• to the printing direction) by a DLP 3D printer (Rasdent SP, Rapid Shape, Raspart, Netherlands) using a denture base material (FREEPRINT denture, Detax, Ettlingen, Germany). Specimens were cleaned in isopropanol > 98% with an ultrasonic cleaner (GT Sonic, China) for 2: 3 minutes, then polished with an automatic polishing system machine (SS1000 Grinder/Polisher; LECO Corp) using a 1200-grit abrasive to provide a uniform and smooth surface.

Yeast was normally cultured for 24 hours at 37° C in a yeast peptone-dextrose medium (YPD medium; Fisher Scientific). YPD medium containing 5 ml of C. albicans {American Type Culture Collection (ATCC 10239)}. The 3D printed acrylic specimens were sterilized in 70% ethanol for 5 minutes, washed in clean water, and then placed on sterile 6-well tissue culture plates in order to evaluate the metabolic activity. Every well included three specimens, 50 ml of yeast culture grown overnight, and 3 ml of YPD. Incubation conditions were 5% CO_2 and 37° C. The medium was discarded after 48 hours and replaced with new YPD. This new solution comprised 8 mg/ml of nicotine and caffeine, with the exception of control samples, which contained 0 mg/ml of each. A method originally reported by Pierce et al. and modified by Li et al.[9,10] was used to determine the metabolic activity of C. albicans. This procedure involved making 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H- tetrazolium- carboxanilide (XTT), which was then filtered, aliquoted, and kept at -70 C.

The plates were then filled with the XTT and menadione solution and left to incubate in the dark at 37° C for two hours. The Spectra Max ABS spectrophotometer from Molecular Devices Corporation was used to measure the colour change in the plates at 490 nm.

The 3D-printed acrylic resin specimens were fabricated using the same method as for measuring metabolic activity, removed from the wells, and placed in a sterile 15-ml tube. Centrifuge tube containing 1 ml of sterile saline to measure the biofilm attachment. After 10 seconds of sonication and 10 seconds of vertexing, the adhering biofilm was moved. On blood agar plates, the dislodged biofilm underwent spiral plating at two dilutions (1:100 and 1:1000). 48 hours were spent incubating the plates at 37° C with 5% CO2. An automated colony-counting device (Protocol 3; Symbiosis) was used to count the yeast colonies and calculate the CFU/ml.

Data were analyzed using SPSS computer program version 25.0. Quantitative data was expressed as means ± standard deviation. Qualitative data was expressed as number and percentage. ANOVA was used to compare the effects of caffeine concentration and the presence or absence of nicotine on differences in metabolic activity and biofilm formation. A 5% level was chosen as a level of significance in all statistical tests used in the study.

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RESULTS

Measurement of biofilm metabolic activity of established C. albicans biofilm in the absence or present of 8.00mg/mL of nicotine were assessed; we evaluated the capacity of yeast to reduce XTT. There was a statistically significant difference ($p<0.05$) between all groups and control group regarding the color changes. While regarding no of colonies (the CFU/ml) the was no significant difference between control group and ((0/8), (0.25/8)and (0.5/8)) while for remaining subgroups there was a significant difference ($p<0.05$).

Absorbance (490nm)

	Mean	+	SD	F	P value	95% CI
0/0	.41	+	.03	832.324		
0/8	.70	+	.06		0.0001	0.2498 to 0.3302
0.25/8	1.06	+	.06		0.0001	0.6098 to 0.6902
0.5/8	1.88	+	.14		0.0001	1.3843 to 1.5557
1/8	2.28	+	.12		0.0001	1.7959 to 1.9441
2/8	1.66	+	.09		0.0001	1.1932 to 1.3068
4/8	1.32	+	.08		0.0001	0.8588 to 0.9612
8/8	.88	+	.01		0.0001	0.4511 to 0.4889
16/8	.68	+	.03		0.0001	0.2446 to 0.2954
32/8	.37	+	.02		0.0009	-0.0616 to -0.0184

P value was calculated by one way ANOVA test

Log CFU

	Mean	+	SD	F	P value	
0/0	6.89	+	.38	7.06		
0/8	6.80	+	.40		0.5777	-0.4203 to 0.2403
0.25/8	6.72	+	.38		0.285	-0.4917 to 0.1517
0.5/8	6.67	+	.36		0.159	-0.5334 to 0.0934
1/8	6.42	+	.48		0.0143	-0.8365 to -0.1035
2/8	6.34	+	.48		0.005	-0.9165 to -0.1835
4/8	6.22	+	.49		0.001	-1.0412 to -0.2988
8/8	6.02	+	.49		0.0001	-1.2412 to -0.4988
16/8	6.01	+	.70		0.0009	-1.3568 to -0.4032
32/8	5.77	+	.67		0.0001	-1.5811 to -0.6589

P value was calculated by one way ANOVA test

DISCUSSION

Wearing dentures can reduce the passage of oxygen and saliva to the soft tissues beneath the denture base, leading the oral cavity environment to become acidic and anaerobic, which promotes the overgrowth of Candida. [11]

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Candida-associated denture stomatitis is identified in 67% of denture wearers, and It has been shown that C. Albicans is the primary yeast related with denture stomatitis. This form of oral illness can affect the patient's quality of life, and treatment needs the denture wearer's participation in the careful daily cleaning of oral tissues and prostheses and the maintenance of oral health.[8] Consequently, Caffeine and nicotine concentrations utilised in this study are consistent with normal intake levels.

The data suggest that denture wearers who smoke tobacco include a risk group for denture irritation in which C. Albicans is implicated, and that they may benefit from a normal caffeine consumption. Caffeine suppresses the biofilm development and metabolic interest of C. albicans, hence decreasing the risk of prosthetic stomatitis in patients. In this study, even at the extremely high nicotine exposure level of 8,000 mg/ml, only a surprisingly low concentration of caffeine was necessary to inhibit biofilm development, and at a little higher concentration, metabolic activity became detected. The most significant external risk factor for oral illnesses such as cancer and periodontal disease is smoking. The main cause of highly progressing periodontitis is a diminished immune response due to the harmful consequences of smoking.[12] In order to lower their risk of denture stomatitis, edentulous denture wearers who also consume caffeine must be encouraged to quit smoking.

According to studies, denture stomatitis affects over 70% of denture users, necessitating careful devotion to oral health hygeine.[13] Therefore, it can be hypothesised that daily consumed beverages may affect denture stomatitis and its caustic components. Caffeine is a chief aspect of coffee. Another study reinforced, using multiple techniques, that caffeine has a prominent direct antifungal property against *C. albicans.* Additionally, our findings demonstrated for the first time the influence of caffeine on *C. albicans* adherent to 3D Printed Denture.

In accordance with the present results, previous research has shown that the biofilm formation and metabolic activity of C. albicans diminish with increasing caffeine concentrations in the presence of 8 mg/mL of nicotine, notably from 0.5 to 32 of caffeine. According to prior investigations, caffeine decreased cell proliferation and caused gene segregation in C. albicans grown on defined complete medium, with both effects rising proportionally with increasing caffeine concentrations.[14]

CONCLUSIONS

The following conclusions were reached based on the results of this in vitro study:

1) The presence of 8 mg/mL of nicotine alone increased the metabolic activity and biofilm formation of C. albicans on 3D Printed Acrylic resin .4
2) When compared with the 0 mg/mL of caffeine and 8.00 mg/mL of nicotine control group, caffeine from 0.25 to 32.00 mg/mL significantly increased C. albicans biofilm metabolic activity.

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3) When compared with the 0 mg/mL of caffeine and 8.00 mg/mL of nicotine group, 0.25, 0.50, 1.00 mg/mL of caffeine significantly decreased the biofilm formation of C. albicans.

Conflict of Interest

There was no conflict of interest.

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