Evaluation of \textit{C. albicans} Adhesion and Growth on Restorative Dental Materials Enriched or not with Fluoride

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Abstract

\textit{Candida albicans} (\textit{C. albicans}) is the most prevalent fungus in the human oral cavity and has been known as the primary cause of denture-related stomatitis. \textit{Candida} cells have a high adhering potential to dental material in almost the same manner as to oral tissues, and they are known to form biofilm that leads to \textit{C. albicans} resistance against antifungal drugs. The aim of this study was to investigate \textit{C. albicans} adhesion and growth on different restorative dental materials and studied the effect of fluoride on \textit{C. albicans} growth and morphological transition. To this end, \textit{C. albicans} (Sc 5314) was cultured on acrylic resins, composite resin, and glass-ionomer materials. Growth was analyzed at various times using scanning electron microscopy analyses and cell proliferation assay. The effect of different concentrations (50 and 100 ppm) of exogenous fluoride on \textit{C. albicans} growth and yeast-to-hyphae transition was investigated.

Scanning electron microscopy showed that \textit{C. albicans} adhered to all of the tested restorative materials. Adhesion was greater on diamond D and ivocap than on composite resin and glass ionomer. After 1 to 4 days, \textit{C. albicans} growth on acrylic resins was two folds that of the composite resin and the glass-ionomer. The latter also displayed the lowest adhesion and growth which may be due to the release of antimicrobial molecules such as fluoride present in this material. This hypothesis is supported by our results showing that exogenous fluoride significantly inhibits \textit{C. albicans} growth and its morphological changes from blastospore to hyphal form. This study clearly demonstrates that restorative materials are conducive to \textit{C. albicans} adhesion and growth. Exogenous fluoride was also shown to down-regulate \textit{C. albicans} growth and morphological changes. Overall data suggest the possible integration of fluoride into dental materials which may control oral microbial pathogenesis.

Keywords: Dental materials; \textit{C. albicans}; Fluoride

Introduction

Multiple structures such as the mucosa and the teeth contribute to the overall function of the oral cavity. Furthermore, restorative materials are frequently used to replace non-functional elements in the oral cavity [1,2]. All available surfaces in the oral cavity are in contact with a plethora of microorganisms that include a large number of bacteria and yeasts. The presence of these microbes on all accessible surfaces of the mouth is both natural and essential for the normal development of the physiology of the oral cavity [3]. The resident oral microflora contributes to the health of the host by preventing exogenous microbial infection, and regulates the inflammatory host response to oral commensal bacteria [3]. Under certain circumstances, however, commensal microorganisms may lead to oral diseases such as caries and candidiasis [4]. Pathogenesis of commensal oral microorganisms is favored by their continuous presence in the oral cavity, profiting by the host immune deregulation. The continuous presence of microorganisms in the oral cavity is promoted by their adhesion to the different surfaces including restorative dental materials. Glass ionomer cements have wide spread clinical indications, being used as temporary and restorative dental materials [5]. The reasons for such a useful clinical versatility are related to the constant fluoride release [6], adhesion to dental tissues and base metals [7], biocompatibility and low coefficient of thermal expansion [8]. The composite resins are also currently used for dental restoration. According to the natural features, the composite resins used for dentin buildup are fabricated and are characterized by lower translucency, whereas the composite resins for enamel are highly translucent [9]. In an attempt to reproduce the enamel properties, high-, medium, and low-value resin composites were introduced, and they are increasingly used for stratified restorations using direct composite resins [10]. Following their introduction in the oral cavity, these restorative dental materials could be additional surfaces for bacterial adhesion and biofilm formation which often lead to local and systemic infections [11].

Biofilm formation is a stepwise process initiated by the adhesion of planktonic bacteria on every surface in the oral cavity, whether these are natural or dental materials [12,13]. This process progresses from colonization and co-adhesion via growth and maturation to the detachment and spreading of the microorganisms from the biofilm [14,15]. In biofilm formation, early colonization is considered to be the most important step, depending on the nature of the host surface.

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It is now well documented that a bacterium adheres directly to the saliva coated host surface before exhibiting extensive inter- and intra-gener coaggregations [16]. Thus the physical and chemical properties of the surface materials may affect the feasibility of bacterial infection [17]. To prevent biofilm formation and host infection, surfaces of restorative materials must therefore harbor the appropriate physical and chemical properties.

Correlations between bacterial adhesion and various surface characteristics (chemical composition, surface energy, surface roughness, and the presence of functional groups on the surface) have been studied in an effort to reduce bacterial adhesion through surface modification [18,19]. Adequate finishing and polishing of restorative materials, such as composite resins, is a prerequisite for high-quality aesthetics and enhanced longevity of resin-based restorations [20]. Smooth, highly polished restorations are easier to maintain than are restorations with a more roughened surface. They are also less susceptible to plaque accumulation and extrinsic discoloration, and display improved mechanical properties [21-23].

Other initiatives were undertaken to prevent microorganism adhesion and growth and biofilm formation, including the incorporation of antimicrobial molecules [24,25], such as fluoride into restorative materials [26,27]. Fluoride modifies the structure of dental hard tissues by increasing the resistance to acids and imparting antibacterial properties to prevent the growth of harmful bacteria and the formation of biofilm [28,29]. Glass-ionomer, a restorative material containing fluoride, was shown to have an inhibitory effect on the growth and adherence of oral bacteria which was attributed to the release of fluoride [30].

Bacteria are not the only microorganisms inhabiting the oral cavity. Yeasts such as C. albicans are frequently encountered in the mouth [31]. C. albicans is the most prevalent yeast isolated from the human body as an opportunistic pathogen [32], and has been widely associated with the etiology of denture related stomatitis [33,34]. C. albicans can adhere to the denture surface and can forms biofilms [35]. It is well known that biofilm cells typically exhibit increased resistance to antifungal agents and the host immune defence [36,37]. The addition of anti-candidal molecule such as histatin-5 to denture acrylic showed Candida growth reduction and reduces the formation of biofilms [38]. Our first aim is to determine the adherence and growth of C. albicans on the surfaces of four different restorative materials such as composite resin (Filtek ™ Supreme Plus), acrylic resins (diamond D, Ivocap) and glass-ionomer (GC Fuji II LC capsule). Our second aim is to investigate the effect of fluoride on C. albicans growth and yeast-to-hyphae morphological changes.

**Experimental Procedures**

**Preparation of restorative material disks**

Capsule of glass-ionomer restorative material GC FujiII (GC America Inc., Alsil, IL, USA) was shaken after mixing the powder and liquid components. The capsule was then immediately activated with Vari-mic ™ II Caulk (Dentsply International Inc., Toronto, ON, Canada) and the material was poured into one well of a 96-well plate to produce glass-ionomer disks (6 mm diameter and 4 mm thickness). This step was repeated as much as required to obtain the appropriate number of disks. The poured material in each well was light-cured for 30 s on each side (top and bottom) using a visible light curing device (3M ESPE Dental Products, London, ON, Canada). Following polymerization, the disks were polished as described below. Resin composite disks were prepared using a Filtek TM Supreme Ultra resin composite syringe (3M ESPE). Resin composite in the syringe was poured into the wells of a 96-well plate at a 160 mg/well and prepared as recommended by the manufacturer.

**Acrylic resin sample preparation**

Two categories of acrylic resin (Diamond D and Ivocap) restorative materials were used in this study.

**Diamond D specimens**

Acrylic resin disks 12 mm in diameter were customized. To do so, a mixture of regular plaster (Kerr Corporation, Orange, CA, USA) was prepared by mixing 100 g of stone powder with 45 ml of water and pouring the mixture into a half flask. A 12 mm diameter cylindrical form was placed into this half flask prior to stone drying. The other half of the flask was prepared by using 100 g of hydrock rapid stone (Kerr Corporation) with 30 ml of water. This was assembled with the first one and left to dry. Thereafter, the cylindrical form was removed, leaving an empty mould. This dry stone mould was then invested with auto-polymerized acrylic resin (Diamond D™, Keystone Industries, Cherry Hill, NJ, USA). Diamond D material was prepared by mixing 2 g of methylmethacrylate (Keystone) with 10 ml of a solution containing methyl-methacrylate, ethylene glycol dimethacrylate, with monomethyl ether hydroquinone as an inhibitor. This mixture was placed into the mould and the flask was then pressed with a hydraulic press (Herbst hydrofix, Brenner Goldschlager, West Germany) at 100 kp/cm² for 15 min. This step was repeated twice to remove excess materials. The resin in the flask was then immersed in a 77°C hot water bath for 90 min, and then transferred to a second water bath at 100°C for 30 min, after which time the flask with acrylic resin was cooled down at room temperature. The acrylic resin specimen was subsequently polished and 3 mm-thick cylindrical specimens were prepared.

**Ivocap specimens**

Acrylic resin disks 12 mm in diameter were also custom made. To do so, a stone mould was prepared as described in the Diamond D acrylic resin preparation. This dry stone mould was then invested with auto-polymerized acrylic resin. The Ivocap material was prepared by mixing the contents of one capsule containing 20 g polymer and 30 ml of monomer (Ivoclar Vivadent Inc. Mississauga, ON, Canada). This mixture was then injected into the mould and the flask was pressed with a hydraulic press (Herbst hydrofix; Brenner Goldschlager) at 6 kp/cm² for 10 min. The flask were then immersed in a 100°C hot water bath for 36 min, after which time the flask containing the acrylic resin was then cooled down at room temperature. The Ivocap acrylic resin specimens were removed from the flask and polished and 3 mm-thick cylindrical specimens were prepared.

**Specimen polishing**

Specimens of each restorative material were assigned to a sequential polishing procedure. The procedure consisted of three steps which were based on the sequential application of SiC papers (Struers Inc. Canada, Mississauga, ON, Canada) having #800, #1200 and #4000 grit. To reduce variability in the specimen preparation, a single operator advance and carried out the polishing procedure in sequence as previously reported [39]. After completing each polishing step using a specific grit, the specimens were extensively washed in PBS to remove any debris, after which time they were sterilized 24 h by incubation in penicillin (250 mg/ml) and streptomycin (10 mg/ml) solution, and then washed extensively with sterile PBS to completely move out antibiotics.
C. albicans growth

*C. albicans* (Strain SC5314) was used in this study. Strain SC5314 was chosen because of its widespread and increasing use in molecular analyses, virulence in animal models, and apparent standard diploid electrophoretic karyotype [40]. *C. albicans* (SC5314) was inoculated into 10 ml of proteinase induction medium (PIM) containing 2% glucose, 0.1% KH2PO4, 0.05% MgSO4, was dissolved in deionised water and adjusted to pH 4.0 (Difco; Becton-Dickinson), and was grown to the stationary phase overnight at 30°C in a shaking water bath. Following culture, blastoconidia were collected, washed in PBS, counted by means of a hemocytometer, and adjusted to 10^6 cells/ml prior to use.

Assessment of *C. albicans* adhesion and growth on the different restorative materials

The tests were performed on 24-well plates. Using a sterile technique, each material disk was placed in the bottom of a well and exposed to 10^6 *C. albicans* in 2 ml of PIM. Following incubation at 37°C for 24, 48, 72, and 96 h, the test materials were transferred to new wells in a 24-well plate and were washed three times with 2 ml of sterile PBS in order to remove the non-adhering cells. Samples were subsequently used for scanning electron microscopy or for *C. albicans* growth assessment.

Scanning electron microscopy

To investigate the ultra-structural *C. albicans* adhesion and growth on the dental materials, each specimen was rinsed in PBS, fixed in 4% paraformaldehyde in PBS for 15 min, and rinsed four times in distilled water. Dehydration was performed in a series of ethanol solutions of increasing concentrations (50, 70, 90, and twice at 100%), with a 5-min dehydration treatment in each solution and a 5-min interval between treatments. The dehydrated specimens were kept overnight in a vacuum oven at room temperature, after which time they were sputter-coated with gold and examined/photographed with a JEOL 6360 LV SEM (Soquelec) operating at a 30 kV accelerating voltage. The experiment was repeated four times and representative photos were taken.

MTT assay

*C. albicans* growth on the restorative dental materials was determined using the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide)] test, which is an established spectrophotometric measurement [41]. The MTT reading is proportional to the number of viable cells. The material disks populated with *C. albicans* were cultured in the presence of 1% (v/v) MTT solution (5 mg/mL) for 4 h, after which time the supernatant was removed and 1 mL of HCl in isopropanol (0.04N) was added to the well. Fifteen minutes later, 200 μL (in triplicate) of solution was transferred from each well to a 96-well flat-bottom plate, and the absorbance of the MTT (formazan) was determined at 550 nm using an ELISA reader (Model 680, BioRad Laboratories, Mississauga, ON, Canada).

Effect of pre-washes of glass-ionomer cement on *C. albicans* growth

Glass-ionomer disks were placed in a 24-well plate and covered with 3 ml of PBS. This solution was changed each day during 7 days. Washed and non-washed glass-ionomer disks were then seeded with 10^6 *C. albicans* in 3 ml of PIM medium. Following incubation for 24 h, *C. albicans* growth was investigated by using a MTT assay, (n = 4).

Effect of exogenous fluoride on *C. albicans* growth

*C. albicans* was grown to the stationary phase (18 h at 30°C) in a shaking water bath. The blastoconidia were collected and washed with PBS, and the suspension was adjusted to 10^6 cells/ml. PIM containing *C. albicans* (10^6 cells) was incubated with fluoride (50 and 100 ppm) for 24 h at 30°C under shaking water conditions. *C. albicans* cultured without fluoride was used as a negative control. *C. albicans* cultured without fluoride but in the presence of 0.5 μg/ml of amphotericin-B (Sigma-Aldrich, Oakville, ON, Canada) was used as a positive control.

Fluoride concentrations were chosen based on previous studies [42,43]. Following an incubation period, an MTT assay was performed to determine *C. albicans* growth. The results are presented as the means ± SD, (n = 4). Following *C. albicans* incubation with fluoride, the cell morphology was analyzed by scanning electron microscopy.

Effect of fluoride on *C. albicans* morphological changes

To determine the effect of fluoride on the yeast-to-hyphae transition, *C. albicans* (10^6 cells) was grown in 3 ml PIM containing 10% FCS. Fluoride was added at different concentrations (50 and 100 ppm). Control *C. albicans* cultures with and without FCS were also performed. The cultures were incubated at 37°C for 3 and 6 h then photographed. Photos were used to record the yeast-to-hyphae transition and to quantify hyphal size. This was measured using an NIH image analyzing program (Image-J Software). The results are the means ± SD, (n = 6).

Statistical analysis

Experimental values are expressed as means ± SD. The statistical significance was determined by means of a one-way ANOVA. Posteriori comparisons were done using Tukey’s method. Normality and variance assumptions were verified using the Shapiro-Wilk test and the Brown and Forsythe test, respectively. All of the assumptions were fulfilled. P values were declared significant at ≤0.05. Data were analyzed using the SAS version 8.2 statistical package (SAS Institute Inc., Cary, NC, USA).

Results

Candida adhesion and growth were dependent on the restorative dental material used

Figure 1 presents the results of the *C. albicans* adhesion and growth on the restorative dental materials. *C. albicans* adhesion after 24 h of culture was more visible on both the resin acrylic-Dimond D and the Ivocap compared to the composite and glass-ionomer cement materials. In addition, the higher level of *C. albicans* adhesion was obtained with low polishing grit. Indeed, more individual *C. albicans* and aggregates were observed on the surface of the materials polished with #800 grit then on those polished with #4000 grit. Growth measurement by means of MTT assay confirmed the SEM analyses (Figure 2) showing a high number of viable *C. albicans* on the surface of the Dimond D and Ivocap resin acrylic materials and a low level on the composite and glass-ionomer materials. This specific growth on the material was maintained at 48, 72, and 96 h. A high growth rate was maintained with the acrylic resin at each of these culture periods compared to the composite and glass-ionomer samples (Figure 1 and Table 1). The reduced adhesion and growth levels on the glass-ionomer, despite its porous shape, may have been the result of the fluoride enrichment. To validate this hypothesis, glass-ionomer disks were washed during 7 days, with medium changing every 24 h. *C. albicans* was subsequently
cultured on the washed and non-washed glass-ionomer cements. As shown in Figure 3, a significantly higher level of C. albicans adhered to and grew on the washed materials, in contrast to the non-washed materials. This indirectly confirms the possible effect of fluoride on reducing C. albicans adhesion and growth.

**Fluoride affects C. albicans growth**

Exposure to fluoride reduced C. albicans growth (Figure 4). After 24 h, the fluoride had significantly, and in a dose-dependent manner, reduced C. albicans growth. Indeed, optical density, which is related to viable cell number, dropped from 0.45 in the non-treated Candida culture to 0.3 with 50 ppm of fluoride and to 0.2 with 100 ppm of fluoride. After 48 h, the inhibitory effect of fluoride on C. albicans growth was even greater than at 24 h, despite no significant differences between fluoride concentrations (50 and 100 ppm) on C. albicans growth after 48 h of treatment. Overall data demonstrates the efficacy of fluoride against C. albicans growth.

**Fluoride affects C. albicans ultrastructure**

Ultrastructural examination of C. albicans showed typical yeast cells displaying the characteristic bud scars (Figure 5) No development of pseudohyphae (chains of elongated non-separated blastospores) was observed in the cultures with or without fluoride or amphotericin B. Following the addition of fluoride, the external morphology of the cells did not appear as smooth as that of the untreated cells, which indicates a possible loss of cytosolic volume. Indeed, fluoride basically distorted the cell wall surface at high concentrations (Figure 5d). It should be noted that the membrane shape of C. albicans cultured in the presence of fluoride (Figures. 5c and 5d) resembled that observed in the presence of amphotericin B (Figure 5b), which suggests that fluoride and amphotericin B had a comparable effect on the C. albicans membrane. Further studies are needed to confirm this observation.

**Fluoride affects C. albicans yeast-hyphal transition**

As fluoride affected C. albicans growth, we investigated its possible effect on the yeast to hyphae transition. As shown in Figures 6A and 6B, fluoride significantly reduced the number of hyphae compared to the control (non-treated Candida cultures). The cultures were maintained for 24, 48, 72, and 96 h at 37°C. Following each culture period, an MTT test was performed on each sample.
culture not treated with fluoride. In the presence of fluoride at 50 and 100 ppm, however, hyphae size decreased dramatically. The effect was observed at 3 and 6 h post-treatment. Overall data shows that fluoride affected both the number of yeast-to-hyphae form and the size of the hyphae.

**Discussion**

Restorative dental materials are commonly used for teeth restoration. In the oral cavity, these restorative dental materials are in contact with multiple microorganisms, including *C. albicans*, which may take advantage of the presence of these dental materials to adhere, proliferate, and may cause oral candidiasis. Adhesion and growth of *C. albicans* may be related to the physico-chemical properties of the material [44-46]. In this study, we analyzed the difference of adhesion and growth of *C. albicans* on four different restorative dental materials. These materials were subjected to the same polishing protocol prior to use. Data shows that the *C. albicans* adhered well and proliferated when in contact with the Diamond D and Ivocap resin acrylic surfaces. These observations are in accordance with those previously reported with different acrylic resins [47,48]. The high adherence of *C. albicans* to acrylic resin may be due to the surface physico-chemical properties. Indeed, it has been reported that the adhesion of various microorganisms, including *C. tropicalis*, *C. glabrata*, *C. dubliniensis*, and *C. albicans* could be promoted by the surface free energy values,
as hydrophobic microorganisms appear to adhere more to acrylic surfaces [49]. With an acrylic resin preparation protocol, it is possible to modulate material surface wettability through acid-base interactions [50]. Through this wettability, the adhesion of microorganisms such as C. albicans may be promoted [51], which supports the data presented in our study. Further investigations are required to elucidate the mechanisms involved in C. albicans adhesion to acrylic resin but not to other dental materials such as composite resin. This study demonstrated a low level of C. albicans adhesion and growth using the Filtek TM Supreme Ultra syringe. These data are in accordance with those reported with comparable material and with S. mutans adhesion and growth [52]. The reduced C. albicans adhesion to the composite material tested in our study may be due to the surface roughness, including the absence of pores. It is well documented that the surface roughness of intraoral hard surfaces has a major impact on bacterial adhesion and retention. Rougher surfaces such as crowns, implant abutments, and denture bases retain more plaque than do smoother ones [53,54]. In patients with inadequate oral hygiene, the surface roughness of provisional restorations is sometimes associated with the onset of subclinical and clinical inflammation [55,56], while smooth intraoral structures ensure patient comfort and facilitate oral hygiene [57]. This may not, however, apply to all restorative material.

In the present study, we demonstrate low adhesion and proliferation levels of C. albicans when cultured onto a rough, porous glass-ionomer material. Our hypothesis is that even under favorable physical conditions (non-smooth, porous material); the reduced level of C. albicans adhesion and growth is due to the chemical composition of the glass-ionomer used. As previously reported, glass-ionomer contains fluoride, a chemical component. Fluoride present in the dental material may act as antimicrobial agent. In vitro studies have shown that amalgam and glass-ionomer may possibly inhibit the growth of cariogenic bacteria and the cariogenicity of oral biofilms [58,59]. An in vivo study using 20 volunteers showed that glass-ionomer material provided protection against secondary caries [60].

To confirm the role of fluoride on C. albicans growth inhibition on glass-ionomer, we activated fluoride release from this material during 7 days, after which time we investigated C. albicans adhesion/growth. Our data revealed both high adhesion and growth on the fluoride-depleted glass-ionomer materials compared to the non-depleted ones. This confirms that the inhibition of C. albicans adhesion and growth on glass-ionomer may be due to the presence of fluoride. To support this hypothesis, we tested the efficacy of exogenous fluoride against C. albicans pathogenesis. Our results show that fluoride reduced C. albicans growth and the yeast-to-hyphae morphological transition. In C. albicans, hyphal formation is known to promote virulence by several mechanisms including invasion of epithelial cell layers [61], breaching and damaging endothelial cells [62], and following phagocytosis. C. albicans hyphal growth can promote lysis of macrophages and neutrophils [63].

Although several groups have investigated the efficacy of exogenous fluoride against bacterial growth and biofilm formation [64,65], this study is the first to investigate the direct effect of fluoride on C. albicans pathogenesis. It therefore suggests the possible integration of fluoride in all restorative dental materials as an antimicrobial agent to prevent microorganism adhesion, growth, and biofilm formation. Further studies will be required to determine the integration techniques, long-term efficient concentrations, and the molecular mechanisms involved in the antimicrobial effect of fluoride. In conclusion, using various restorative dental materials, this study shows that C. albicans adhesion and growth is specific to each of these materials, as even with facilitating physical properties, the glass-ionomer surface showed low yeast adhesion and growth levels. This may be due to the presence of fluoride, as this exogenous component was able to control C. albicans growth and morphological transition.

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References

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