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ORIGINAL ARTICLE

Investigation of the optimal condition for the growth and biofilm development of *Candida albicans* on three dental materials

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ABSTRACT

Background and Objectives: *Candida albicans* as pathogenic fungi cause conditions like oral candidiasis and dental caries. The critical role of biofilms in the pathogenicity of *C. albicans* necessitates the exploration of conditions that promote their growth and development. Our study aimed to delineate the optimal conditions conducive to the proliferation and biofilm production of *C. albicans* on prevalent dental materials.

Materials and Methods: To approximate oral cavity conditions, culture media were enhanced with various glucose concentrations to assess the growth and biofilm-forming capability of the fungus through growth curve analysis and crystal violet assays.

Results: The findings suggest that YPG medium augmented with 4% glucose presents as an optimal environment for *C. albicans* growth. Biofilm formation was most effectively promoted in RPMI medium supplemented with the same concentration of glucose. Composite resin was identified as the substrate most susceptible to biofilm development by *C. albicans* under these conditions.

Conclusion: This investigation highlights the necessity of accounting for microbial activity and material characteristics in the prevention and management of dental biofilm formation. Our research advances the understanding of in vitro cultivation of *C. albicans*, simulating the oral milieu more accurately and contributing to enhanced oral health management for individuals utilizing temporary dental fixtures.

Keywords: *Candida albicans*; Fungi; Culture; Condition; Growth; Biofilm; Dental materials

INTRODUCTION

Candida albicans, an opportunistic fungal species, is a significant constituent of the human oral microbiota, coexisting with a diverse microbial population including *Streptococcus mutans*, a bacterium implicated in the etiology of dental caries (1, 2). This symbiotic relationship suggests a contribu-

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tory role of *C. albicans* in carcinogenesis, thereby challenging the exclusive bacterial paradigm traditionally held responsible for carious lesions. The prevalence of oral candidiasis attributed to *C. albicans* is estimated to be between 18.5% and 40.9% among healthy individuals, with a noted increase in incidence among immunocompromised populations such as HIV-positive patients, individuals with diabetes mellitus, those undergoing specific pharmacological treatments, neonates, and geriatric cohorts (3-8). *C. albicans* is implicated in the pathogenesis of dental caries through its involvement in dental biofilm formation and acidogenic activity, leading to the demineralization of dental enamel (9-11). The adherence of *C. albicans* to dental surfaces is a critical factor in biofilm development, augmenting carious processes by fostering increased plaque formation, dysbiosis, and enhanced pathogenicity (12, 13).

Investigative studies have elucidated the propensity of *C. albicans* to adhere to various dental materials, including but not limited to prosthetic devices and orthodontic appliances, thus underscoring the role of these substrates in fungal colonization. Significant adherence of *C. albicans* to acrylic resin used in denture bases and its capacity to integrate within multispecies biofilms have been documented, indicating that the topographical and mechanical properties of these materials may modulate fungal adherence and biofilm resilience, potentially impeding antimicrobial interventions (14-16). Notwithstanding the extensive body of research investigating the proliferative and biofilm-forming dynamics of *C. albicans* on dental materials, there remains a lacuna in our understanding of the precise conditions that emulate the oral milieu conducive for its growth. Culture media, providing vital nutrients for experimental investigations into the biological behavior of *C. albicans*, are instrumental in modeling its growth dynamics. Glucose supplementation has been observed to influence *C. albicans* phenotypic transition, metabolic activity, and biofilm morphogenesis (17, 18). Nevertheless, there is a paucity of comparative analyses examining the differential impact of glucose across varied culture media on *C. albicans* growth kinetics and biofilm architecture. The present study endeavors to delineate the optimal experimental parameters to promote *C. albicans* biofilm development, thereby contributing significantly to the domain of oral health research.

MATERIALS AND METHODS

Microbial strain. *Candida albicans* (ATCC 10231), conserved at -80°C, was reactivated through incubation in Brain Heart Infusion (BHI) broth at 37°C under aerobic conditions. A fungal suspension was then prepared by adjusting the optical density to OD_{530} = 0.1, equivalent to 10^8 CFU/mL, using a spectrophotometer. The suspension was standardized to McFarland 0.01 in BHI, creating a uniform experimental baseline.

Growth curve. The experimental setup aimed at studying the factors influencing the growth pattern of *C. albicans* is outlined in Fig. 1. To analyze *C. albicans* growth dynamics, the study employed a variety of culture media, including peptone, BHI, and Yeast Peptone Glucose (YPG), with different glucose concentrations from 1% to 4%. Additionally, growth under both static and shaking conditions at 37°C was investigated. Optical density at OD_{530} was measured for quantitative analysis of growth in the respective media, with triplicate measurements ensuring data validity.

Biofilm formation. *C. albicans* was cultured to an initial density of 10^6 CFU/mL and incubated at 37°C for 24 hours in BHI. The resulting suspension was diluted to achieve a concentration conducive to biofilm development. This process was conducted in a 96-well polystyrene plate with 200 µL of inoculated suspension and BHI medium, varying in sugar content. Incubation occurred under microaerophilic or microanaerophilic conditions at 37°C for 24 hours, with the final row of wells serving as bacterial growth controls. Replication of the process ensured experimental consistency.

Crystal violet assay (CV assay). The Crystal Violet (CV) assay was utilized to assess biofilm formation post a 24-hour incubation. Planktonic cells were removed, and the wells were rinsed with saline at 37°C. Plates were dried and treated with 0.1% crystal violet solution, washed with PBS, and then dried again. Absolute alcohol was used to solubilize the stained biofilms, with the absorbance read at 595 nm for quantitative assessment.

Statistical analysis. Statistical analysis was performed on the absorbance data to identify the most

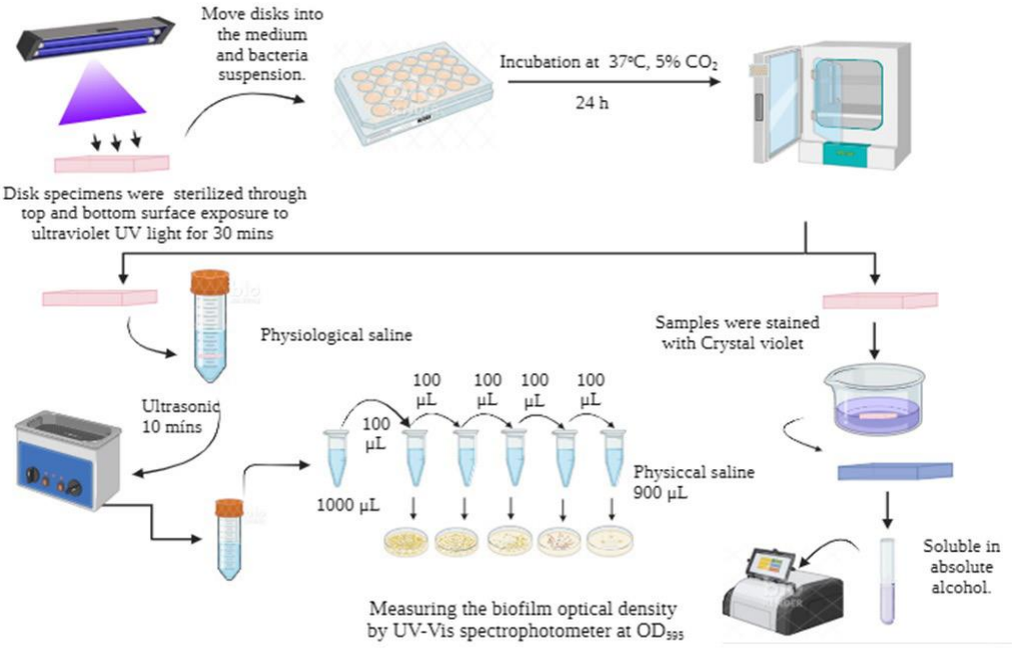

Fig. 1. Schematic procedure for investigating the biofilm formation of *Candida albicans* on dental materials including platics, composite resin and tempofit

conducive conditions for growth and biofilm development. Variations in conditions were discerned using one-way analysis of variance (ANOVA), with Tukey's post-hoc test for detailed comparison, and a significance level set at $P \leq 0.05$. Biofilm growth percentages were computed relative to the control, and two-way ANOVA, along with Tukey's test, was applied for comparison of these percentages, maintaining the same significance threshold.

RESULTS

The inclusion of glucose in peptone enhanced the proliferation of *Candida albicans.* In the cultivation of *Candida albicans*, peptone-based media is indispensable, with glucose supplementation being investigated for its effect on growth. The fungus was incubated in peptone media with gradients of glucose concentration (1-4%) and monitored over 48 hours. Without glucose, *C. albicans* reached the logarithmic phase at 8 hours and stationary phase by 12 hours (Fig. 2A). Glucose addition markedly enhanced the growth rate, initiating the log phase at 5 hours and extending to the stationary phase by 24 hours under static conditions, signifying that glucose accelerates *C. albicans* proliferation (Fig. 2A). Higher glucose concentrations directly correlated with increased fungal development at the 48-hour checkpoint (Fig. 2B). This pattern persisted under shaking conditions, with a notable increase in growth rate at 4% glucose concentration, indicating that shaking in conjunction with high glucose levels substantially fosters *C. albicans* growth (Fig. 2C, D, E). Thus, glucose is a potent enhancer of *C. albicans* growth in peptone media, with agitation amplifying this effect.

The addition of glucose in BHI promoted the growth of *Candida albicans.* Brain Heart Infusion (BHI), a comprehensive medium employed for microbial cultures, is instrumental in studying *Candida albicans.* BHI medium's ability to replicate the human host environment is critical for analyzing *C. albicans*' physiological and pathological characteristics. The addition of glucose to BHI was analyzed to understand its effect on *C. albicans* growth under static and shaking conditions. Growth curves indicated that the fungus entered the logarithmic growth phase after 12 hours and the stationary phase at 24 hours under static conditions (Fig. 3A). The inclusion of glucose markedly propelled fungal growth, with the 48-hour assessments revealing that higher glucose concentrations significantly bolstered development (Fig. 3B). Under agitation, the growth pat-

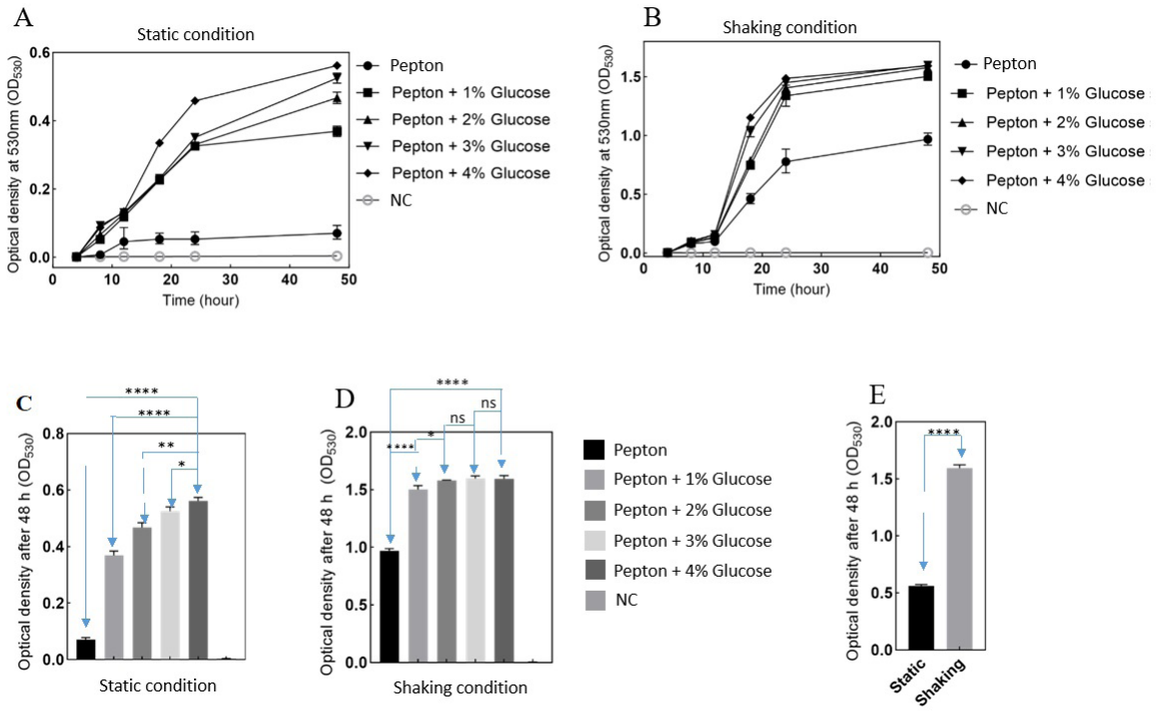

Fig. 2. The influence of glucose supplementation in peptone culture medium on the growth of *Candida albicans*. (A) Growth curves were generated for *C. albicans* cultured in peptone medium with varying glucose concentrations (1% to 4%) under static conditions, with observations recorded at 5, 8, 12, 18, 24, and 48-hour intervals over 48 hours. (B) Growth curves were generated for *C. albicans* cultured in peptone medium with varying glucose under shaking conditions. (C) The optical density of *C. albicans* growth was measured after 48 hours in the presence of glucose under static conditions. (D) Optical density measurements were taken under shaking conditions. (E) Optical density readings after 48 hours of culturing with 4% glucose under both static and shaking conditions were compared. Significant differences (*P < 0.05) were noted, indicating the impact of glucose concentration and culture conditions on *C. albicans* growth. NC: negative test. ns: no significant.

terns were consistent with static observations, and increased glucose concentrations, especially 2-4%, notably supported growth (Fig. 3C, D, E). These findings corroborate that glucose supplementation in BHI medium under dynamic conditions is advantageous for *C. albicans* development, underscoring the medium's utility in mimicking host conditions and fostering microbial growth.

The supplementation of glucose in YPG medium promotes the growth of *Candida albicans*. The Yeast Peptone Glucose (YPG) medium, rich in nutrients with peptone, yeast extract, and glucose, is pivotal for the growth of *Candida albicans*. Chosen for its ability to trigger specific metabolic pathways, YPG facilitates the study of fungal behavior in a host-like environment. Investigating the glucose influence on *C. albicans*, the medium was supplemented with varying glucose levels and subjected to static and agitated growth conditions. Growth trajectories in YPG with 1% glucose paralleled those in YPG alone, while higher glucose proportions notably spurred fungal proliferation (Fig. 4A, B). Elevated glucose concentrations correlated with enhanced *C. albicans* development, with the most pronounced growth seen at 4% glucose, especially under shaking conditions (Fig. 4C, D, E). These results emphasize that higher glucose levels in YPG significantly promote *C. albicans* growth, suggesting the importance of glucose concentration in optimizing conditions for fungal studies.

YPG with 4% glucose is an optimal culture condition for the growth of *Candida albicans*. The selection of culture media for *Candida albicans* research is dictated by specific experimental aims. Peptone's simplicity affords utility in routine fungal maintenance, while Brain Heart Infusion (BHI) with its nutrient diversity is optimal for physiological explorations. Yeast Peptone Glucose (YPG), with its

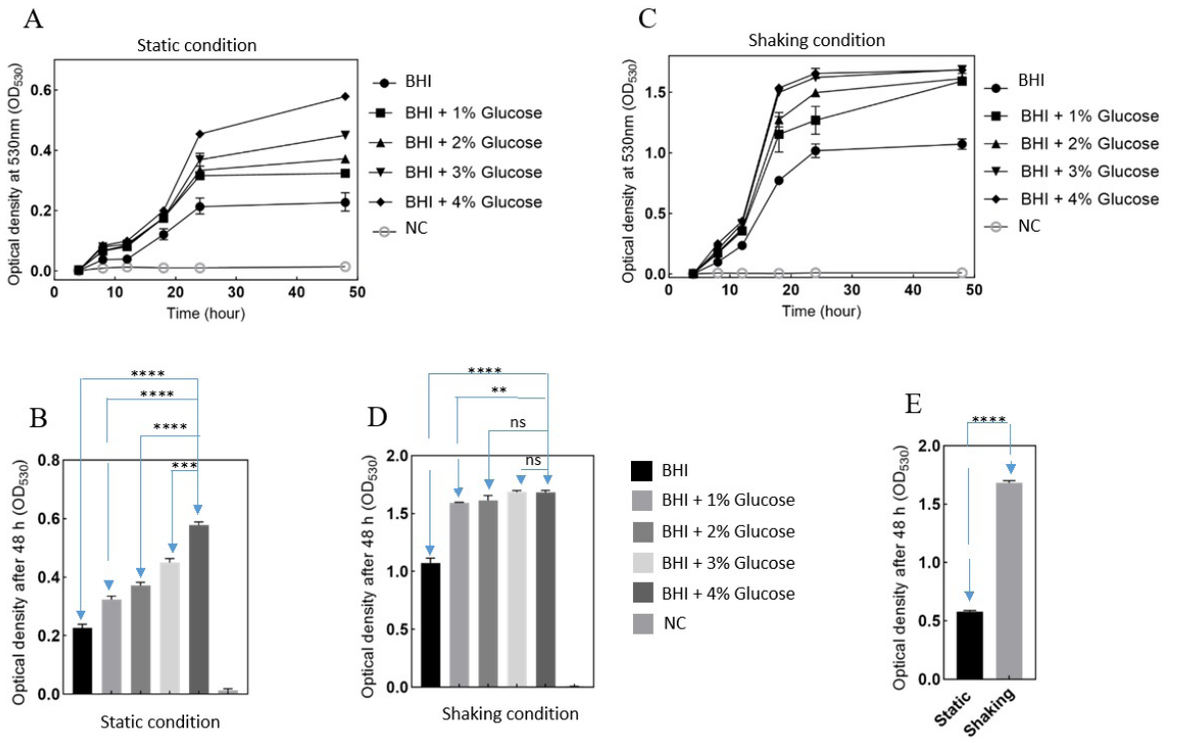

Fig. 3. The impact of glucose addition in BHI culture medium on the growth of *Candida albicans*. (A) The growth of *C. albicans* in BHI medium supplemented with varying glucose concentrations (1% to 4%) was assessed. The fungus was cultured statically, and observations were made at 5, 8, 12, 18, 24, and 48-hour intervals over 48 hours. (B) The growth curve of *C. albicans* in BHI medium supplemented with different concentrations of glucose, ranging from 1% to 4%. The fungus was cultured under shaking condition. Observations were recorded at 5, 8, 12, 18, 24 and 48-hour intervals throughout a 48-hour duration. (C) The optical density of *C. albicans* growth was measured over a 48-hour duration in the presence of glucose under static conditions. (D) The optical density of *C. albicans* growth for a duration of 48 hours in presence of glucose under shaking condition. (E) The optical density of *C. albicans* growth was measured after culturing for 48 hours in the presence of 4% glucose under both static and shaking conditions. Statistically significant differences (*P < 0.05) were observed, suggesting the influence of both glucose concentration and culture conditions on the growth of *C. albicans*. NC: negative test. ns: no significant.

rich composition, excels in metabolic studies. When examining *C. albicans* with oral health implications in mind, the choice of medium is aligned with research goals. Glucose enhancement across these media is pivotal, affecting fungal growth variably. Static condition trials indicate 4% glucose in YPG most significantly augments *C. albicans* growth, with peptone and BHI showing less differentiation (Fig. 5A). Under agitated conditions, the same glucose concentration yields higher growth in YPG than in peptone or BHI (Fig. 5B). Thus, YPG enriched with 4% glucose, particularly under dynamic conditions, is identified as the superior medium for promoting robust *C. albicans* growth.

The impact of glucose supplementation in BHI, YP and RPMI culture media on the biofilm formation of *Candida albicans*. Biofilm formation is a critical virulence trait of *Candida albicans*, aiding in adhesion, resistance to antifungals, and infection persistence. Investigating biofilm development is essential for thwarting *Candida* biofilm-associated infections. The extent of *C. albicans* biofilm formation is influenced by the choice of culture medium and the presence of glucose. In a comparative study, Brain Heart Infusion (BHI) and RPMI media, supplemented with glucose, significantly increased *C. albicans* biofilm formation across a range of concentrations (Fig. 6A, C). In contrast, Yeast Peptone (YP) medium did not demonstrate a substantial impact on biofilm formation with glucose enhancement (Fig. 6B). Among the media tested with a 4% glucose addition, RPMI markedly outperformed others, with biofilm formation enhanced sevenfold compared to BHI and

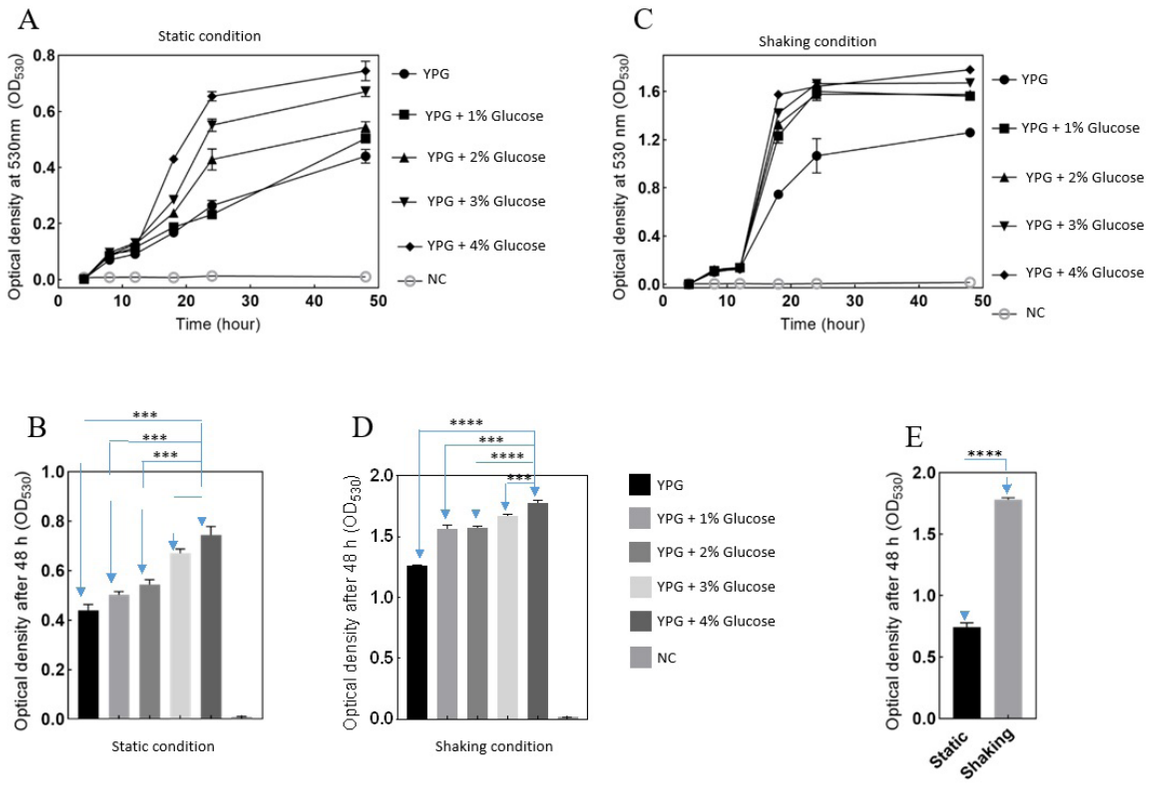

Fig. 4. The effect of glucose supplementation in YPG culture medium on *Candida albicans* growth. (A) Growth curves were plotted for *C. albicans* in YPG medium with varying glucose concentrations (1% to 4%) under static conditions, with data recorded at 5, 8, 12, 18, 24, and 48-hour intervals over 48 hours. (B) The growth curves were generated for *C. albicans* in YPG medium with different glucose concentrations under shaking conditions, with observations recorded at the same intervals. (C) Optical density measurements were taken over 48 hours to assess *C. albicans* growth in the presence of glucose under static conditions. (D) Optical density readings were collected over 48 hours to evaluate *C. albicans* growth with glucose supplementation under shaking conditions. (E) Optical density of *C. albicans* growth was examined after 48-hour culturing with 4% glucose, comparing static and shaking conditions. There were statistically significant differences (*P < 0.05), indicating the impact of both glucose concentration and culture conditions on the growth of *C. albicans.* NC: negative test. ns: no significant.

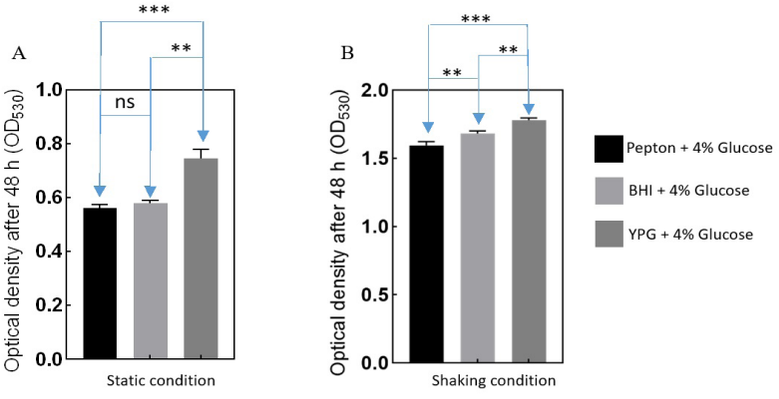

Fig. 5. The optimal condition for the growth of *Candida albicans.* (A) The optical density evaluating the growth of *C. albicans* after 48-hour culture in peptone, BHI and YPG in the presence of 4% glucose under static condition. (B) The measurements of optical density assessing the development of *C. albicans* in 3 different culture medium including peptone, BHI and YPG with 4% glucose supplement over 48 hours under shaking condition. Statistically significant variations (*P < 0.05) were evident, suggesting the influence of glucose concentration and culture conditions on *C. albicans* growth. ns: no significant.

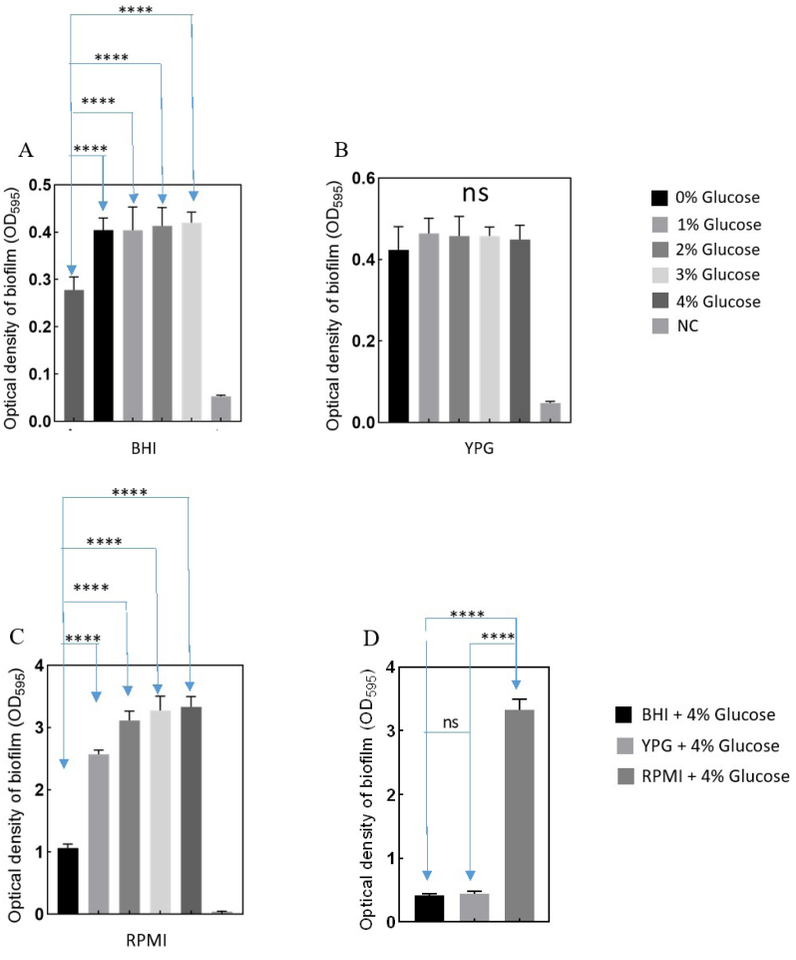

Fig. 6. The influence of glucose addition in culture medium on the biofilm formation of *Candida albicans*. (A) The optical density assess biofilm formation in addition of different concentration of glucose (1% to 4%) in BHI culture medium after 48 hours culture. (B) The optical density indicates biofilm development of *C. albicans* in presence of different concentration of glucose in YP culture medium. (C) Biofilm formation of the fungus in addition of glucose in RPMI culture medium over 48 hours was determined by optical density. (D) Addition of 4% glucose in BHI, YP and RPMI culture medium on the formation of biofilm of *C. albicans*. There were statistically significant differences (*P < 0.05), indicating the impact of both glucose concentration and culture conditions on the growth of *C. albicans*. ns: no significant.

YP, which showed negligible differences (Fig. 6D). Consequently, RPMI medium fortified with 4% glucose is identified as the prime environment for *C. albicans* biofilm maturation.

The impact of incorporating glucose into the culture medium on *Candida albicans* biofilm formation on plastic, composite resin, and tempofit substrates. In modern dental practices, the use of various materials is critical for restorative and prosthetic applications, with plastic, composite resin, and tempofit being particularly prominent. These materials, while beneficial, are prone to microbial colonization and biofilm formation, notably by *Candida albicans*. Plastics are widespread in dental tools and offer flexibility and cost-effectiveness but are susceptible to microbial colonization and subsequent biofilm formation, raising concerns for cross-contamination and infection. Composite resin is lauded for its aesthetic appeal and durability in restorative dentistry but is not immune to microbial adhesion and biofilm establishment. Tempofit, essential for temporary dental solutions, also faces challenges with microbial colonization and potential oral infections. To investigate *C. albicans* interactions with these materials, the fungus was cultivated in RPMI medium enhanced with 4% glucose, creating a condition akin to the oral environment. The resulting colony count

assays indicated a more pronounced biofilm formation on plastic and composite resin than on tempofit, with no significant disparity between plastic and resin (Fig. 7A). However, the biofilm biomass was substantially higher on composite resin than on the other materials (Fig. 7B). These results highlight composite resin's particular susceptibility to *C. albicans* biofilm development, underscoring the need for careful consideration of material properties in the context of dental material selection and maintenance.

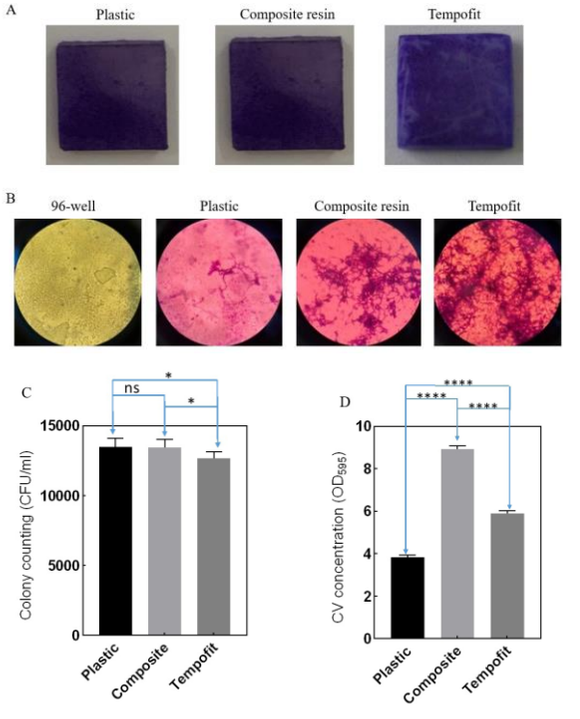

Fig. 7. The biofilm formation of *Candida albicans* in RPMI culture medium supplemented with 4% glucose. (A) Crystal violet staining of *C. albicans* biofilm on the surface of dental materials including plastic, composite resin and tempofit. (B) The biofilm of *C. albicans* on the surface of dental materials under microscope. (C) The colony number of biofilm from *C. albicans* on the surface of plastic, composite resin and tempofit after culturing in RPMI culture medium with 4% glucose. (D) The biomass of *C. albicans* biofilm in RPMI culture medium supplied with 4% glucose on the surface of dental materials.

DISCUSSION

The present investigation reveals a pivotal aspect of *C. albicans* biology by elucidating the fungus's growth responses to varying glucose concentrations across different culture media, shedding light on potential avenues to enhance in vitro cultivation and subsequent analysis of its life cycle. Our findings have relevance to the documented correlation between increased salivary glucose and heightened susceptibility to oral candidiasis (19). The pronounced growth of *C. albicans* in glucose-enriched environments is a critical consideration for dental health, especially among the immunocompromised and diabetic populations, suggesting that glucose intake may drive the proliferation of this pathogen, thus contributing to the onset of dental caries. This underscores the need for comprehensive insights into the dietary glucose sources and their potential oral health risks, advocating for the refinement of dietary guidelines to bolster dental health. Further, our study illuminates the role of glucose in facilitating *C. albicans* biofilm formation—a key virulence attribute in oral infections. Glucose's substantial support of *C. albicans* biofilm development is consistent with its implicated role in the pathogenesis of various oral conditions, such as oral candidiasis, denture stomatitis, and dental caries (20-22). Notably, the enhanced biofilm production in glucose-supplemented RPMI culture medium offers an ideal setting for in vitro studies of *C. albicans*, enabling in-depth investigations into the fungal biofilm's impact on oral health.

Our research underscores the prevalence of glucose in a wide array of foods, encompassing both natural and processed items, and draws a connection with *C. albicans* biofilm development, thereby linking dietary habits to oral health outcomes. It highlights that foods, typically with inherent glucose levels surpassing 4%, are congruent with our findings where such glucose concentrations optimally support *C. albicans* biofilm formation (23-25). This relationship between dietary glucose and microbial biofilm development accentuates the influence of daily food choices on oral health and underscores the importance of targeted preventive measures in dental care. *C. albicans'* ability to form biofilms on dental materials, including plastic, composite resin, and tempofit, presents significant clinical challenges (26-28). These biofilms are robust communities that enhance the pathogen's resistance and virulence. Our study indicates the facilitation of *C. albicans* biofilm formation on these materials in the presence of 4% glucose. The particular propensity of *C. albicans* to form biofilms on composite resin, widely used in dental restorations, indicates an elevated infection risk for individuals with such restorations, reinforcing the

necessity of stringent hygiene practices and infection control measures in dental practices. Prophylactic strategies, such as meticulous disinfection routines, surface treatment to minimize microbial adherence, and antimicrobial application, are vital to prevent *C. albicans* infections and maintain oral health.

Our findings indicate that RPMI medium, when supplemented with 4% glucose, demonstrated a significantly stronger effect on *Candida albicans* biofilm formation compared to BHI and YPG culture media. Previous research has established that RPMI provides a balanced composition of nutrients and amino acids conducive to the yeast-to-hyphal transition, a critical process for mature biofilm development (29). This medium facilitates both hyphal growth and extracellular matrix production, whereas BHI medium is comparatively less effective in supporting filamentation, and YPG medium primarily supports planktonic cell growth but is less favorable for biofilm matrix development (30, 31). Furthermore, biofilms cultivated in RPMI medium are generally denser and exhibit higher metabolic activity compared to those grown in BHI or YPG, as indicated by metabolic assays (32). Farnesol production, a quorum-sensing molecule that regulates biofilm maturation, is also higher in RPMI medium than in YPG, further promoting robust biofilm formation (31). These findings highlight the superiority of RPMI medium in facilitating *C. albicans* biofilm development through enhanced filamentation, metabolic activity, and extracellular matrix production. In contrast, BHI and YPG support biofilm formation to a lesser extent and often require additional supplementation to achieve comparable results. In sum, this research accentuates the need to understand biofilm dynamics on dental materials, which is imperative to devise effective interventions against *C. albicans*-associated oral diseases, thereby enhancing patient outcomes and the quality of dental care.

CONCLUSION

The oral cavity provides an optimal environment for the proliferation of diverse microorganisms, including *Candida albicans*, commonly found as a commensal in individuals using temporary dental materials. To replicate oral cavity conditions relevant to temporary dental material users, we investigated various parameters to determine the optimal conditions for *C. albicans* growth and biofilm formation on materials such as plastic, composite resin, and tempofit. Our findings demonstrate that culturing *C. albicans* with 4% glucose enhances both growth and biofilm formation on temporary dental materials, particularly composite resin. This discovery suggests avenues for future research into natural compounds from medicinal sources in Vietnam that may inhibit *C. albicans* biofilm formation on composite resin fillings. In conclusion, while traditional views attribute dental caries mainly to bacterial pathogens, emerging evidence suggests that *C. albicans* may play a role in the complex microbial dynamics of the oral cavity, impacting dental caries development. Further research is needed to fully understand *C. albicans'* interactions with oral bacteria and its specific role in oral infections and dental caries.

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