



Effectiveness of Resin Denture Containing Surface Pre-Reacted Glass Ionomer (S-PRG) Fillers on Antifungal Property: A Clinical Study

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Abstract

Patients with dentures are exposed to soft tissue inflammation and denture stomatitis. A promising approach to addressing these issues involves incorporating bioactive surface pre-reacted glass ionomer (S-PRG) fillers into denture base resin for their antimicrobial properties. Therefore, this study evaluated the effectiveness of a modified resin denture base containing S-PRG microfillers and nanofillers in inhibiting *Candida* growth among healthy Thai elderly individuals wearing complete dentures. This randomized clinical trial involved 44 participants aged 50–80 years (n=14-16/group); dentures were fabricated in three variations: 0wt% (control), 20wt% S-PRG microfillers, or 10wt% S-PRG nanofillers. Before overnight storage, participants were instructed to apply a 1450 ppm fluoride gel to their dentures. Samples were collected using a swabbing technique at baseline, 1 month, and 3 months after wearing the dentures. Yeast growth was quantified as colony-forming units (CFU), and yeast species were identified using MALDI-TOF MS. Data were analyzed with non-parametric tests (Related-Samples Wilcoxon Signed Rank Test), with statistical significance set at $p < 0.05$. The results showed that *Candida* CFUs significantly decreased from baseline to 1 and 3 months in the group using dentures with S-PRG microfillers. No *Candida* overgrowth was observed in the nanofiller group, while the control group showed an increase in CFUs over time. The most frequently isolated species was *Candida albicans*, followed by *Candida tropicalis*. In conclusion, incorporating S-PRG microfillers and nanofillers into denture base resin effectively inhibits *Candida* growth. *C. albicans* was the predominant species in denture wearers.

Keywords: S-PRG filler, microfiller, nanofiller, *Candida albicans*, *Candida tropicalis*, denture base

1. Introduction

Removable complete prostheses remain a preferred treatment for edentulous patients, as they improve function and oral health-related quality of life. However, denture wearers often experience changes in their oral environment due to compromised salivary flow, which can promote biofilm formation. As a result, denture bases may accumulate for microorganisms, contributing to oral diseases such as denture stomatitis and chronic inflammatory responses in the oral mucosa (Ellis et al., 2007; Mirzadeh et al., 2018; Veyrune et al., 2005). Among denture wearers, *C. albicans* is the most frequently isolated species in the oral cavity, found in 45–65% of healthy individuals. Its prevalence increases to 60–100% in denture wearers, followed by non-*albicans* *Candida* spp. (NAC) such as *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, and *C. krusei* (Lotfi-Kamran et al., 2009; Manikandan et al., 2022). Recent research has focused on incorporating bioactive materials such as metal oxide nanoparticles, mesoporous silica nanoparticles, hydroxyapatite nanoparticles and surface pre-reacted glass ionomer (S-PRG) fillers into polymer denture bases to serve as antifungal delivery systems (Jo et al., 2017; Kaurani et al., 2024; Malakhov et al., 2016; Sun et al., 2013). One promising approach involves incorporating S-PRG fillers into polymethyl methacrylate (PMMA) resin as a denture base material.

S-PRG fillers are inorganic bioactive materials derived from fluoro-boro-aluminosilicate glass (Ito et al., 2011). These fillers consist of three layers: an outermost SiO₂ coating, a pre-reacted glass-ionomer phase, and a glass core by the acid-base reaction. This structure enables ion exchange with the environment through diffusion. S-PRG filler can release six types of ions (F⁻, BO₃³⁻, Al³⁺, Na⁺, SiO₃²⁻ and Sr²⁺) and have ion storage capabilities, allowing them to release and recharge ions depending on environmental

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concentration (Jitaluk et al., 2022; Mayumi et al., 2021). Many products containing S-PRG filler are already available on the market, such as resin composites, adhesives, resin cement, coating resins, fissure sealants, polishing pastes, and temporary fillings (Imazato et al., 2023). Due to the release of F^- and other ions, S-PRG filler exhibits antifungal properties. Previous studies have shown that fluoride can inhibit the growth of bacterial and fungal cells, suggesting its effectiveness in reducing the biomass of mature biofilms by disrupting the integrity of cell membranes (Binder et al., 2019; Flisfisch et al., 2008; Li & Breaker, 2012; Pointer et al., 2015). Even low concentrations of boric acid have demonstrated inhibitory effects on *Candida*, *Trichophyton*, and various bacteria, causing cytoskeletal disintegration in inhibiting *microorganisms* (Liu et al., 2021; Syvolos et al., 2024). The studies have confirmed that PMMA resin containing S-PRG microfillers can inhibit the growth and adhesion of *C. albicans* on resin surface, reducing the risk of denture stomatitis. It is hypothesized that the release of BO_3^{3-} and F^- ions from S-PRG microfillers can inhibit the yeast-to-hypha transition, thereby preventing biofilm development (Tsutsumi et al., 2016). Moreover, a previous study reported that the release of ions from S-PRG fillers induced oxidative stress, which suppressed adhesion, hyphal transformation, and protease production in *C. albicans*, thereby inhibiting fungal growth and pathogenicity (Tamura et al., 2018).

Nanofillers, defined as invisible particulate substances with diameters between 1 and 100 nm, possess a large surface area-to-volume ratio, which makes them uniquely effective as delivery and antimicrobial agents. Over the past few decades, nanofillers have been applied in various fields to enhance human health. Their properties, such as size, shape, charge, and surface area, determine their activity (Moraes et al., 2021; Sharmin et al., 2021). Recently, S-PRG nanotechnology has been introduced and initially evaluated for its potential applications in dentistry. S-PRG nanofillers, with their fine grains, have a higher surface area-to-volume ratio than microparticles, enabling enhanced ion release and significantly greater fluoride release (Jitaluk et al., 2022; Mayumi et al., 2021). S-PRG nanofillers are now being explored for a new fluoride delivery system to prevent caries, reduce fungal adhesion, and delay biofilm formation (Tonprasong et al., 2021). Another study investigated the incorporation of various concentrations of S-PRG microfillers (10% and 20%) and nanofillers (2.5%, 5%, 10%, 20%, and 30%) into a tissue conditioner to evaluate their efficacy in reducing *C. albicans* adhesion. The findings revealed that S-PRG nanofillers increased surface roughness and altered the material's surface characteristics. Nonetheless, they significantly reduced candidiasis by decreasing the number of living cell adhesions more effectively than the 20 wt% microfillers (Tonprasong et al., 2021). However, The inhibitory effects of incorporating S-PRG micro/nanofillers into PMMA denture base resin on *Candida* growth have not yet been supported by evidence from clinical studies. Therefore, we conducted this study to investigate the influence of incorporating S-PRG fillers into PMMA denture base resin on inhibiting *Candida* growth among healthy Thai elderly individuals wearing complete dentures.

2. Objectives

- 1) To investigate the effect of incorporation S-PRG micro and nanofillers in the resin denture base in inhibiting *Candida* growth in Thai elders aged 50-80 years wearing complete dentures.
- 2) To identify the yeast species related to wearing complete dentures containing S-PRG micro and nanofillers in Thai elders aged 50-80 years.

3. Materials and Methods

This study was a 1-year, intervention-control, randomized clinical trial involving edentulous subjects aged 50–80 years who required complete dentures. The Institutional Review Board of Thammasat University reviewed and approved the study protocol before implementation. A sample size of 36 subjects was calculated to generate 80% power for detecting differences in CFU/ml based on a previous study (Tsutsumi et al., 2016). Allowing for a possible 20% dropout rate, a total of 44 subjects, with 14 in each group, were required for recruitment. The colony-forming units per millilitre (CFU/ml) were measured at baseline, 1 month and 3 months after wearing a denture. Yeast species were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS).

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Recruitment

A total of 44 edentulous subjects were recruited from the Student Clinic at the School of Dentistry, Thammasat University. Subjects were randomly assigned to one of three study groups, with each group receiving dentures made from resin containing S-PRG filler at concentrations of 0wt%, 20wt% microfillers, or 10wt% nanofillers. Block randomization was used to assign a numeric ID to each subject. Both participants and clinical staff were blinded to group assignments until the study concluded.

Inclusion and exclusion criteria

Subjects were eligible for inclusion if they were Thai citizens who could read and write, were cooperative throughout the research period, were in good general health, were capable of performing basic self-care, and had fully edentulous ridges. Subjects who exhibit signs of xerostomia (unstimulated saliva flow rate < 0.5 ml/min), a history of allergy to resin, a disability that could interfere with study participation, having denture stomatitis, receiving medication, or having medical conditions that predispose or promote to oral candidiasis such as uncontrolled diabetes mellitus, anemia or immunodeficiency disorders were excluded from the study.

Screening process

The screening process involved an interview and an assessment of unstimulated saliva flow using a split test over 1 minute. Eligible subjects who meet the inclusion criteria were informed about the study details and provided written informed consent before participation.

Intervention

Complete denture treatment was performed following standard protocol by dental students under the supervision of academic advisors at the Prosthodontics Clinic, School of Dentistry, Thammasat University. At the try-in stage, all dentures were sent to a participating commercial dental laboratory for fabrication as part of routine procedures. A notation on each script indicated to the laboratory whether the corresponding denture was to be made with PMMA resin (URBAN RESIN[®], Shofu Inc., Kyoto, Japan), PMMA resin containing 20 wt% S-PRG microfillers (URBAN RESIN[®] with S-PRG microfillers, particle size 3.0 µm, Shofu Inc.), or PMMA resin containing 10 wt% S-PRG nanofillers (URBAN RESIN[®] with S-PRG nanofillers, particle size 0.48 µm, Shofu Inc.). The polymer powder of the PMMA denture base with added S-PRG micro/nanofillers was prepared by Shofu Inc., Kyoto, Japan, through mechanical blending.

At the delivery visit, subjects completed a questionnaire covering general demographic information. Samples were collected using the swab technique and oral rinse technique at baseline (before denture delivery), 1 month, and 3 months of wearing dentures. Sterile swab sticks were wiped in a circular motion across the edentulous mucosa and inserted into individual sterilized tubes before being transported in a sealed storage box to the Microbiology Laboratory, School of Dentistry, Thammasat University. The samples were spread on chromogenic agar and incubated at 37 °C for 24-48 hours to examine yeast growth. Each type of yeast colony was selected and subcultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 24 to 48 hours. The Sabouraud dextrose agar plates were then transferred to the Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, following the Standard Operating Procedure (SOP) for the identification of yeast species. Fresh growth of yeast colonies was extracted using the formic acid extraction method and identified using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

For the oral rinse sampling method, Subjects were required to gargle a 0.45% normal saline solution for 1 minute and then split into a sterilized test tube. It was crucial that participants must abstain from consuming water, eating food, and brushing their teeth for at least 1 hour prior to sample collection. When more than 300 yeast colonies were found, the oral rinse samples were centrifugated at 1500 rpm for 5 minutes. The clear supernatant was discarded, and the remaining precipitate was resuspended in 1 ml of phosphate-buffered saline (PBS, pH 7.2). After this step, 900 µl of PBS was mixed with 100 µl of the resuspended



solution. From this mixture, 100 μ l was spread onto a chromogenic agar plate for quantified as colony-forming units (CFU).

At the delivery visit, dentures were individually fitted and adjusted for comfort. Standard home care instructions were provided, emphasizing during the days of wearing. Participants were instructed to clean their dentures with water according to the routine procedure for denture wearers. Additionally, they were directed to apply 1 gram (1 cm ribbon of toothpaste) of fluoride toothpaste (1450 ppm, Colgate Palmolive, Thailand) on the denture surface before storing the dentures in a storage box overnight while sleeping. Participants were also advised to thoroughly rinse the dentures with water to remove the fluoride toothpaste before daily reinsertion.

The subjects were recalled for a check-up and adjustments to ensure the comfort of wearing the dentures after 1 week. These follow-up visits were repeated at 1 month and 3 months after wearing the dentures, with samples collected using the same sampling techniques.

Statistical analysis

Data were collected and analyzed using IBM SPSS Statistical software version 26. The average colony-forming units per ml (CFU/ml) were analyzed using non-parametric tests (Related-samples Wilcoxon Signed Rank Test). Statistical significance was considered at a *p-value* of less than 0.05.

4. Results and Discussion

4.1 Results

A total number of 44 subjects with a mean age of 66.44 years participated in the study. They were randomly assigned to 3 groups to receive complete dentures containing S-PRG fillers at 0wt%, 20wt% microfillers, and 10wt% nanofillers. After 3 months of follow-up, 36 subjects (12 per group) remained in the study, resulting in a drop-out rate of 16.28% (7 subjects; see participants flow diagram, Fig.1). No subjects experienced broken dentures during the study, and no harm or adverse reactions were reported. The baseline demographics of the subjects across the 3 study groups showed no statistically significant differences, except for oral rinse carriage (Table 1).

Table 1 Baseline demographics of the participants

	0wt% of S-PRG	20wt% microfiller	10wt% nanofiller	<i>p</i> value
Gender (%)^a				
Male ^a	7 (58.33)	6 (50.00)	5 (41.67)	0.717
Female ^a	5 (41.67)	6 (50.00)	7 (58.33)	
Average age (years)^b	64.58 \pm 5.47	69.25 \pm 5.69	65.50 \pm 4.96	0.095
Smoking^a				
Yes (or quit smoking less than 3 months)	10 (83.33)	11 (91.67)	9 (75.00)	0.549
No	2 (16.67)	1 (8.33)	3 (25.00)	
Oral yeast carriage (Swab)^a				
Present	7 (58.3)	10 (83.3)	4 (33.3)	0.046*
Absent	5 (41.7)	2 (16.7)	8 (66.7)	

a. Data are presented as n (%), and *p* values are based on a chi-square test.

b. Data are presented as the mean \pm SD, and *p* values are based on a one-way ANOVA test.

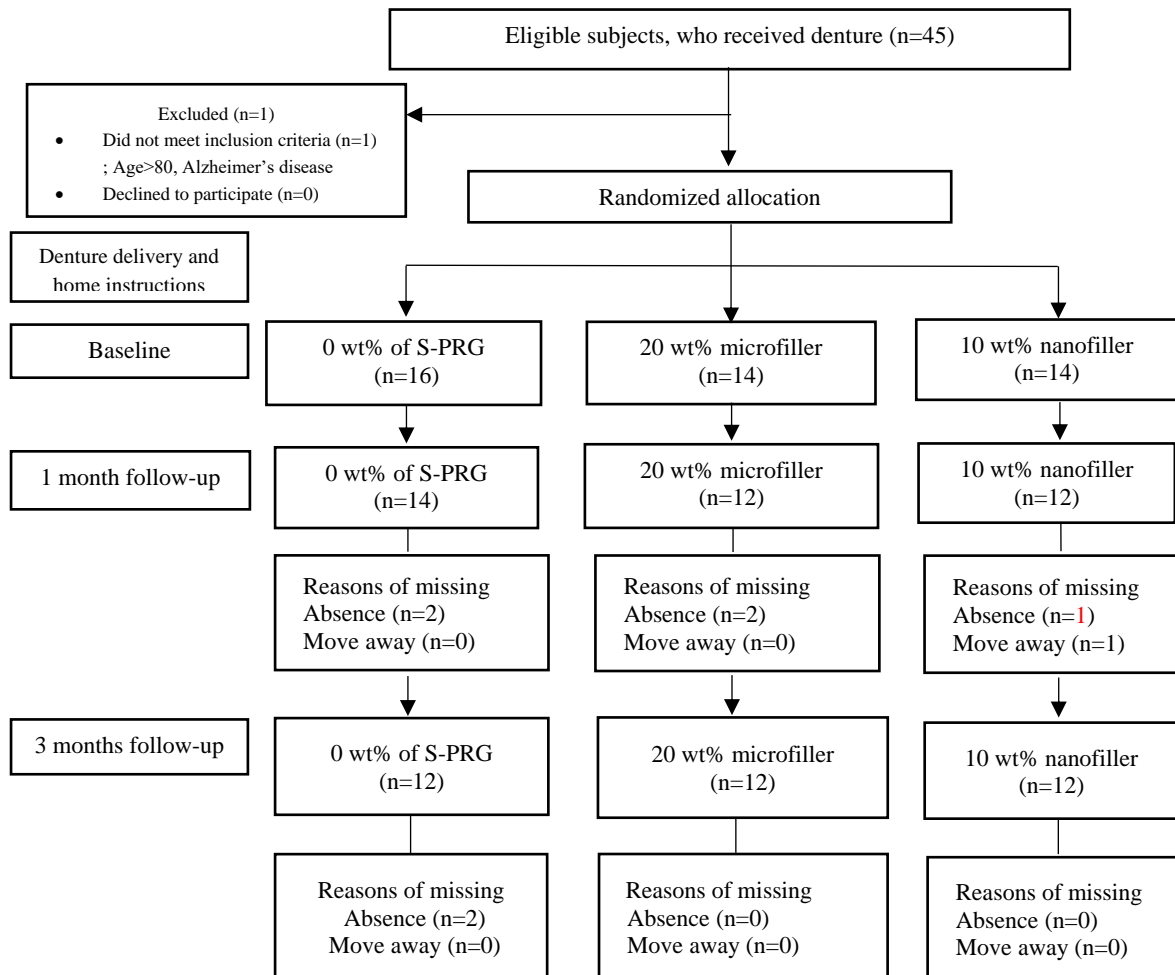


Figure 1 Participant flow diagram

At baseline, the median number of *Candida* was detected for 5, 4.5, and 0 CFU/ml in patients wearing resin dentures containing 0wt%, 20wt% microfillers, and 10 wt% nanofillers of S-PRG, respectively. The median number of *Candida* in the control dentures significantly increased to 22.5 CFU/ml after wearing the dentures for 3 months. In contrast, the median number of *Candida* was undetectable in subjects who wore the S-PRG microfiller dentures for 1 and 3 months. Similarly, *Candida* was not detected in participants wearing the nanofiller dentures at baseline, but it remained stable and undetectable after wearing the dentures for 1 month and 3 months. The median CFU/ml (IQR) of *Candida* growth observed from swabbing at baseline, 1, and 3 months of wearing dentures is presented in Table 2.

The odds ratio of the *Candida* growth

The number of subjects was determined to compare the outcomes of arrests and increased rates of *Candida* growth (Table 3-4). The study found that 67% of the subjects in the control group developed *Candida* growth after 3 months of wearing the denture, compared to 25% for the 20 wt% microfiller of S-PRG and 33% for the 10 wt% nanofiller of S-PRG. Therefore, individuals wearing the control denture without the S-PRG filler were 2.8 times more likely to experience progression in *Candida* growth than those wearing the 10 wt% S-PRG nanoparticle denture and 4.2 times more likely than those wearing the 20 wt% S-PRG microparticle denture.



Table 2 The median (IQR) CFU/ml of *Candida* growth and *p*-value for the comparison between baseline and 1 month of follow-up, baseline and 3 months of follow-up from swab technique

Grop	Baseline	Follow-up 1 month	Follow-up 3 month	<i>p</i> -value	<i>p</i> -value
	Median (IQR)	Median (IQR)	Median (IQR)	Baseline Vs Follow-up 1 month	Baseline Vs Follow-up 3 months
Owt% of S-PRG	5(0-20.76)	3(0-15.75)	22.5(3.25-89.25)	1	0.241
20Wt% microfiller	4.5(1.25-43)	0(0-2)	0(0-2.25)	0.005*	0.008*
10Wt% nanofiller	0(0-4)	0(0-4.5)	0(0-2.25)	0.715	0.686

Note: A symbol * represents a significant difference between baseline and follow-up time points at $p < 0.05$.

Table 3 shows the number of subjects with a decrease, stable, or progression in the amount of *Candida* growth in group 0 wt% of S-PRG, 20 wt% microfiller of S-PRG and 10 wt% nanofiller of S-PRG when comparing the day of denture delivery and three months after wearing dentures from swab technique.

Variable	Group	Arrested (decrease or stable) of <i>Candida</i> growth	Progress of <i>Candida</i> growth	OR (95% CI)
Baseline Vs Follow-up 3 Months	0 wt% of S-PRG filler (control)	5	7	4.2(0.74-23.9)
	20 wt% microfiller of S-PRG	9	3	
Baseline Vs Follow-up 3 Months	0 wt% of S-PRG filler (control)	5	7	2.8(0.5-14.7)
	10 wt% nanofiller of S-PRG	8	4	

Identification of yeast using MALDI-TOF MS

After identifying the yeast species, *C. albicans* was found to be the most common species (40%) isolated, followed by *C. tropicalis* (11%). Non-*Candida* species, including *Lodderomyces elongisporus* (5%), *Trichosporon asahii* (5%), and *Myerozoma caribbica* (3%) were also commonly detected. However, we observed the growth of Gram-negative bacteria on Oxoid Chromogenic *Candida* Agar, with the most prevalent species being *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

When we observed the number of cases with *C. albicans* in microfiller group, we found that the number of cases with *C. albican* decreased by 71.42% from the day of denture delivery to 3 months after wearing the dentures. Additionally, cases with non-*albicans Candida* species decreased by 83.33%, and cases with non-*Candida* species declined by 50% during the same period. In the nanofiller group, *C. albicans* was still detected 3 months after denture use; however, the number of cases did not increase from the day of denture delivery. In contrast, the number of subjects with *C. albicans* in the control group increased over time. Additionally, cases with non-*albicans Candida* species decreased by 66.67% after 3 months of denture wear.

4.2 Discussion

The null hypothesis of inhibiting the growth of *Candida* was rejected. The results of this study suggest that resin denture bases containing S-PRG microfillers effectively reduced *Candida* growth after one and three months of denture wear. Additionally, resin denture bases with S-PRG nanofillers demonstrated

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potential in inhibiting *Candida* growth throughout the study, as suppressed *Candida* growth was observed from the day of denture delivery to three months of wearing dentures. In contrast, the control denture without S-PRG fillers exhibited a significant increase in *Candida* growth. This effect was probably due to the capability of multi-ions (Na, Si, Sr, Al, B and fluoride) release capability of the S-PRG material (Fujimoto et al., 2010; Imazato et al., 2023; Nakatsuka T, 2003).

Boric acid, a broad-spectrum antimicrobial agent, is widely used to treat fungal infections. Previous studies have indicated that boric acid, even at low concentrations, boric acid can inhibit *C. albicans* growth by destabilization fungal membranes. This mechanism involves reduced ergosterol content and interference with hyphal transformation through cytoskeletal disruption (Gavilanes-Martínez et al., 2021; Pointer et al., 2015). Boric acid has demonstrated therapeutic effects in treating cutaneous *C. albicans* infections and has recently been incorporated into clinical guidelines. Its antibacterial properties against various yeasts effectively limit the growth of *C. albicans* and prevent smooth *C. albicans* proliferation (Liu et al., 2021).

Fluoride is well-documented for its ability to inhibit bacterial and fungal cell growth, by disrupting the function of key metabolic enzymes. Studies have shown that sodium fluoride (NaF) exhibits fungicidal activity at high intracellular concentrations (Tascioglu et al., 2017). Sodium can induce high osmotic stress that kills *C. albicans* cells while increasing their doubling time (Flisfisch et al., 2008). Clinical studies involving elderly nursing home residents reported that using a mouthwash and toothpaste containing amine fluoride–stannous fluoride (AmF–SnF₂) twice daily significantly reduced the proportion of subjects with high *Candida* counts after 8 months of follow-up. These findings suggested that the use of a topical AmF–SnF₂ could potentially support therapeutic treatment for oral candidiasis (Meurman et al., 2009). The antifungal activity of AmF/SnF₂ against virous oral *Candida* species is believed to result from its interaction with yeasts plasma membranes, similar to the action of chlorhexidine. Moreover, this study concluded that AmF/SnF₂ mouthrinse provides the dual benefit of antifungal and anti-plaque effects (Meurman et al., 2006). In addition to fluoride ion release from S-PRG filler, fluoride dentifrices (1450 ppm) were used in this study to recharge fluoride levels in the dentures. The sustained fluoride release from S-PRG microfillers and nanofillers may enhance the antimicrobial properties of complete dentures and contribute to the long-term effectiveness of fluoride in preventing candida growth.

When examining *Candida* growth in the nanofiller group, no increase or detection of *Candida* was observed at baseline, after one month, or three months of denture wear. This outcome may be attributed to the higher proportion of non-smoking participants in this group compared to the others. We propose that proper oral and denture hygiene maintained by the subjects and the antifungal properties of S-PRG nanofillers contributed to the absence of *Candida* growth during all follow-up periods in the nanofiller group. Previous studies have confirmed that smoking promotes the growth and adhesion of *candida* cells, potentially leading to *C. albicans* biofilm formation (Ye et al., 2021). Sardari et al. (2021) reported that cigarette smokers have an increased odds ratio for denture stomatitis. This association could be related to increased candida carriage, as smoking increases epithelial thickness and changes the functional activity of the keratinocytes, which leads to candida colonization (Sardari et al., 2021). Additionally, several studies have reported that poor denture hygiene can stimulate the development of denture stomatitis by allowing plaque and biofilm to accumulate on the palatal denture surface, creating an environment conducive to bacteria and fungi growth. This makes the mucosa more susceptible to infections, such as denture stomatitis (Aoun & Cassia, 2016). Thus, maintaining proper oral and denture hygiene and refraining from smoking are crucial in preventing *Candida* growth. Additionally, incorporating S-PRG nanofillers into PMMA denture base resin at a concentration of 10 wt% enhances the bio-functional properties of the material. This incorporation provides a fungicidal effect, significantly reducing the risk of denture stomatitis.

After identifying *Candida* species using MALDI-TOF MS with colonies on chromogenic agar, we found that the predominant *Candida* species was *C. albicans*. Other types of yeast were found, such as *C. tropicalis* and *C. glabrata*. Moreover, the present study revealed that the S-PRG microfiller has the potential to decrease the number of subjects with *C. albicans* and non-*albicans* *Candida* species, when observed from the day of denture delivery to three months of wearing dentures. The group with S-PRG nanofiller did not lead to an increase in the number of subjects with *C. albicans* after wearing dentures for 3 months. However,

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factors such as routine use, operator's manual skills, polishing equipment, and abrasive materials may affect the surface roughness of dental prostheses.

The roughness of the resin denture base is a key factor contributing to increased candida growth. Previous studies have reported that the surface porosity and roughness of acrylic resin are directly related to the accumulation of microorganisms deposited on the surface, which influences the formation of biofilms (Alhadj et al., 2024). Al-Fou-Zan et al. confirmed that the presence of such irregularities, porosity, or imperfections on the surface of a dental device enhances microbial accumulation, even when it is clean (Al-Fouzani et al., 2017). Moreover, surface roughness enhanced the yeast-to-hypha transition of *C. albicans*, likely attributed to the thigmotropism exhibited by *C. albicans*. When yeast cells come into contact with a surface, yeast cells switch to hyphal growth, and hyphae of *C. albicans* invade the cracks and protuberances on the resin strip. Contact with solid surfaces induces hyphal growth and biofilm formation (Mayahara et al., 2014). Based on these findings, while surface roughness may increase after three months of denture wear, incorporating S-PRG micro/nanofillers into resin exhibits antifungal activity and may effectively reduce *in vivo* biofilm formation by *Candida* species. This makes S-PRG fillers a promising option for clinical use in the near future.

Kamijo et al. (2009) evaluated various concentrations of S-PRG incorporated into a resin denture base and identified 20 wt% as the highest concentration that maintains the mechanical properties required by international standards. (Kamijo et al., 2009). Moreover, another study revealed that incorporating 20 wt% of S-PRG microfillers into denture resin increased saliva fluoride concentrations in adults who wore it for 1.5 years. The pattern of fluoride release from resin containing S-PRG fillers was initially rapid on the first day but gradually decreased over time. The study also reported that sustained fluoride release required routine recharging with a fluoride solution. (Kiatsirirote et al., 2019). In contrast, incorporating S-PRG nanofillers at a 20 wt% concentration enhances fluoride release compared to microfillers but reduces the flexural strength to below the standard requirement of 65 MPa (Jitaluk et al., 2022). Accordingly, the present study uses test specimens with 20% microfiller content and 10% nanofiller content, along with recharging using 1450 ppm fluoride toothpaste, to ensure that the mechanical strength of the denture base exceeds 65 MPa, as required by international standard while maintaining sustained fluoride release over an extended period.

The placement of a prosthesis in the oral cavity significantly alters environmental conditions, as the prosthesis becomes colonized by oral microorganisms and prevents the underlying mucosa from receiving the mechanical cleansing effects of the tongue and the antimicrobial benefits of saliva flow (Chandra et al., 2001; Tamura et al., 2018). Denture stomatitis is a common oral mucosal disease with a global prevalence ranging from 20% to 67%. It develops due to chronic inflammation affecting areas of the oral mucosa beneath a denture, with strains of the genus *Candida* frequently implicated as a causative factor. Over the decades, numerous studies have focused on enhancing the antimicrobial properties of denture base materials to reduce the incidence of denture stomatitis (Abualsaud, & Gad, 2023). Hatano et al. (2021) reported a similar finding, showing that a minimum S-PRG content of 7.5 wt% in denture adhesives is necessary to suppress the growth of *C. albicans*, *C. glabrata*, *S. mutans*, and *A. naeslundii* (Hatano et al., 2021). Based on the results, we observed that incorporating S-PRG micro/nanofillers into resin denture bases reduced the odds ratio of subjects who developed *Candida* growth compared to those with denture base resins without S-PRG fillers. Therefore, incorporating 20 wt% of S-PRG micro and 10 wt% of S-PRG nanofiller significantly inhibits planktonic candida growth before biofilm formation on denture base resin while still maintaining acceptable strength in compliance with international standards. However, future clinical trials are needed to evaluate the effects of S-PRG micro- and nanofillers on *Candida* biofilm adhesion to the resin surface. Furthermore, the long-term efficacy of denture base resins containing S-PRG fillers in reducing *Candida* growth requires further exploration in subsequent research.

In the present study, Gram-negative bacteria colonies grew from 4 samples growing on Oxoid Chromogenic Candida Agar. This finding aligns with the observations of Baixench et al. (2006), who reported that bacterial colonies grew from 11 of the 364 clinical samples, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia fonticola*, *Escherichia coli*, and *Serratia marcescens*. However, Bacteria growth was always limited and never interfered with yeast identification. Finally, the culture of Oxoid



Chromogenic Candida Agar was as efficient as that of CHROMagar for *Candida* growth and produced reliable results (Baixench et al., 2006).

5. Conclusion

Within the limitations of our study, we found that the resin denture base containing 20 wt% of S-PRG microfillers significantly reduced *Candida* growth after three months of wearing dentures. Additionally, the resin denture base with 10 wt% of S-PRG nanofillers demonstrated potential in inhibiting *Candida* growth when compared between baseline and three months of wearing dentures. This suggests a reduced risk of denture stomatitis among denture wearers. MALDI-TOF MS identification revealed that *C. albicans* was the most common species found in the oral environment, particularly among denture wearers.

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