

Candidal Growth in Conventional Heat Cure and Permanent Self Cure Resins.

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Abstract:

Candidiasis is common in denture wearers. *Candida albicans* is a type of yeast that is common member of the human gut flora. Infection of denture materials with *Candida albicans* is common and contributes to denture stomatitis. Thus, this study is being carried out to find which denture is more susceptible to candidal growth and to find a material that is better to maintain by denture wearers. The aim of the study was to compare the growth of *Candida albicans* in permanent denture base materials such as heat cure resin and permanent self cure resin. In the present experimental study, 10 square shaped (10 × 10 × 1 mm) heat cure and self cure resin acrylic plates were prepared (5 samples for each group). The specimens were kept in a flask containing physiological serum (NaCl 0.85%), sterilized in an autoclave at 121°C for 15 minutes and incubated at 40°C for further adherence testing. The resin specimens were immersed in distilled water in order to promote the maximum water sorption to prevent, when in culture, the occurrence of distortion and the release of residual monomer after polymerization. *Candida albicans* were grown and cultured in experimental heat and self cure resins and the slide count and serial dilutional test were done. The growth of *Candida albicans* was more in permanent self cure resin when compared to conventional heat cure resin. There was a statistically significant difference between the levels of colonization of *Candida albicans* between the test groups ($P < .05$). The growth of *Candida albicans* was seen more in permanent self cure resin plates than heat cure resin plates. Thus, even though permanent self cure resin plates are easier to process, conventional heat cure resin is less susceptible to Candidal growth and easier to maintain by denture wearers.

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Candida infections receive increasing attention, presumably due to the increased prevalence worldwide. Numerous studies have shown that several *Candida* species possess a multitude of virulence mechanisms leading to successful colonization and infection of the host when suitable conditions occur. (Quirynen et al., 1990) The recognition that *Candida* is an important pathogen has led to many laboratory studies evaluating these virulence attributes in an attempt to clarify the pathogenesis of the disease. The progress made in understanding some of these features, such as the mechanisms that result in adherence to surface. Fungi normally live as innocuous commensals and colonize various habitats in humans, notably skin and mucosa. Commensal existence of oral *Candida* species varies

from 20% to 50% in a healthy dentulous population. (Kuriyama et al., 2003)

As growth on surfaces is a natural part of the *Candida* lifestyle one can expect that *Candida* colonizes denture (McMullan-Vogel et al., 1999). Substrate surface properties, as surface charge, surface free energy, hydrophobicity, and roughness have all been reported to influence the initial adhesion of microorganisms (Panagoda et al., 1998). Microbial adhesion on biomaterial surfaces depends on the surface structure and composition of biomaterials, and on the physicochemical properties of the microbial cell surface. Components of the resilient denture liners and acrylic resin may reduce the adhesion and inhibit the growth of *Candida* (Panagoda et al., 2001). Higher adherence of particular *Candida* species, e.g. *C. tropicalis*, *C. glabrata* and *C. dubliniensis*, when

compared with *C. albicans*, might be attributed to their relative surface free energy values, since hydrophobic micro-organisms seem to be more adherent to acrylic surfaces. (Kuriyama et al., 2003; Luo & Samaranyake, 2002)

The most common form of oral candidiasis, denture-associated stomatitis, involves biofilm growth on an oral prosthetic surface. Cells in this unique environment are equipped to withstand host defenses and survive antifungal therapy (Abaci, 2011). The presence of saliva is known to change this scenario. The nature of the substratum may influence the formation and the composition of the salivary pellicle, which layer may then become more relevant than the surface properties of the dental material itself (Farah et al., 2002). Liners are needed in many clinical situations in which patients have thin, sharp, or badly resorbed residual alveolar ridges or chronic tissue irritation from dentures.

Even though these materials exhibit excellent tissue tolerance, one of the problems is the colonization of *Candida* spp. on and within the material. Fungal growth is known to destroy the surface properties of the liner and this may lead to irritation of the oral tissues. This is due to a combination of increased surface roughness and high concentrations of exotoxins and metabolic products produced by the fungal colonies (Mothibe & Patel, 2017). The aim of the study was to compare the growth of *Candida albicans* in permanent denture base materials such as heat cure resin and permanent self cure resin.

MATERIALS AND METHODS

Preparation of Resin Plates:

In the present experimental study, 10 square shaped (10 × 10 × 1 mm) heat cure and self cure resin acrylic plates were prepared (5 samples for each group). The

specimens were kept in a flask containing physiological serum (NaCl 0.85%), sterilized in an autoclave at 121°C for 15 minutes and incubated at 4°C for further adherence testing. The resin specimens were immersed in distilled water in order to promote the maximum water sorption to prevent, when in culture, the occurrence of distortion and the release of residual monomer after polymerization.

Culture of *C.albicans*:

Pure cultures of *C. albicans* ATCC 90028 were grown on Agar Sabouraud plates containing 500 mMol/L of sucrose at 25°C. After 24 h, the colonies were suspended in tubes containing 5 mL of brain heart infusion (BHI) broth. Next, the specimens were placed into the tubes containing BHI plus inoculum and remained for 11 h at 37°C in order to favour an initial colonization of the acrylic resin surfaces.

These fungal agents were cultured on Saborraud Dextrose agar at 37°C for 48 hours aerobically and the inoculums of these fungal agents were adjusted to CFU/mL (colony forming unit/mL) in the Saborraud Dextrose Broth according to the turbidometric method. Standardized suspensions of the chosen microorganisms were adjusted to CFU/mL and confirmed by measuring their optical density (OD) spectrophotometrically 625nm. Each acrylic specimen was immersed into test tubes including 10 mL of SDB for *Candida albicans* and were put into an incubator at 37°C for 24 hours. After the incubation, the specimens were discarded from the tubes gently and washed three times with 5 mL amount of phosphate-buffered saline solution (PBS), (pH: 7.2).

Slide Count:

Specimens were placed on a special slide count (Neubauer Slide Counter; Chambers- Marienfeld, Germany) after adding 2.5 µL of Trypan Blue 0.4% solution in phosphate to 7.5 µL of each sample to be evaluated under light microscope. Trypan Blue stain can differentiate between dead and alive *Candida*

albicans cells; dead *Candida albicans* usually appear blue while alive *Candida albicans* appear transparent with a blue peripheral line. To count the number of *Candida albicans*, a light microscope with a magnification of 10× was used. *Candida albicans* were counted in two squares out of the four main squares of the slide count and multiplied by 2 to find out the total number of *Candida albicans* in each slide.

Serial Dilution Test:

A 10 µL of each bullet was taken, and then it was diluted serially and spread on a petri dish containing

Sabouraud dextrose agar and incubated for 48 h at 37 °C. A marker pen counter (Colony Counter) was used to count the number of *Candida albicans* colonies in each quadrant where acceptable growth was noted and the final number was corrected for the dilution factor. If the number of colonies was 500 or more, it was considered as an overgrowth.

RESULTS

Table 1: *Candida albicans* growth in denture bases

Candidal albicans count	Heat cure resin	Self cure resin	P value
Slide count	3014.5±117	5201±213	<.05
Serial dilution test	2018.3± 213	4210.3± 204	<.05

Candida albicans growth was significantly high in permanent self cure resins (Table 1). The results obtained were statistically treated using the independent t test. There was a statistically significant difference between the levels of colonization of *Candida albicans* between the test groups (P<.05).

DISCUSSION

The present study shows that the candidates growth was high in permanent self cure resin in both slide count and serial dilution tests. The increased growth maybe due to the porosity present in the denture resins during processing and varies with different processing methods. However, studies regarding the influence of whole saliva on *Candida* adherence are mutuality contradictory and no consensus can be found in the literature. (Salerno et al., 2011) Several investigators

reported that a saliva coating reduces the adherence of *C. albicans* in acrylic resin based materials, Others showed increased adherence rates with saliva coating. Three other research groups found no effect at all of a saliva coating (Kadir et al., 2007; McMullan-Vogel et al., 1999). *L. acidophilus* did not inhibit the *Candida*; the inoculum grew to a concentration of 6 X 10⁶ organisms/rnl. *E. coli*, one of the organisms that inhibited *Candida*, grew rapidly in saliva with a lag period of 60 min and a population-doubling time of 30 min. In un-sterilized saliva from normal subjects, growth of an inoculum of *Candida tropicalis* (2 X 10³ organisms/rnl) was inhibited and reached only 1 X 10⁴ organisms/rnl in 48 hr. By contrast, growth of the same inoculum in sterile saliva reached a concentration of 2 X 10⁶ organisms/rnl (Kadir et al., 2007; McMullan-Vogel et al., 1999)

The growth of *Candida albicans* was seen more in permanent self cure resin plates than heat cure resin plates. The porosities in permanent self cure resin was comparatively less compared to temporary self cure resin, but the candidal growth did not reduce significantly. (Farah et al., 2002) Thus, even though permanent self cure resin plates are easier to process, conventional heat cure resin is less susceptible to Candidal growth and easier to maintain by denture wearers.

CONCLUSION

The growth of *Candida albicans* was seen more in permanent self cure resin plates than heat cure resin plates. Thus, even though permanent self cure resin plates are easier to process, conventional heat cure resin is less susceptible to Candidal growth and easier to maintain by denture wearers.

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