ANTI-FUNGAL EFFICACY OF PROBIOTICS IN HEAT CURE DENTURES BASE RESIN: AN INVITRO STUDY

Abstract

The aim of the following study is to check whether probiotics alter Candidal adherence onto the denture base material, taking PMMA into consideration in this study. Test samples were fabricated in the following way using a metal die. A die was manufactured that had 10 slots, and each slot was of dimension 10 mm×10 mm and 2 mm thick. Modeling wax was taken in a glass of metal and melted on top of the flame. The melted wax was poured into the slots of the die and allowed to cool down. This particular study was done to evaluate the effect of probiotics on Candidal adherence on denture base resins. Candidal colony cell count was done on heat cure denture base materials both before and after incorporation of probiotics at varying concentrations to the samples and the results obtained were compared and used for statistical analysis. The data available with respect to invivo application of probiotics at varying concentration to prevent candidal adhesion to denture is still scarce and need further investigation and research.

Keywords: probiotics, Candidal colony, colonization, oral microbia

Introduction:

With the increase in life expectancy, geriatric problems became more frequent. The human oral cavity is known to harbor a multitude of organisms. Among them, C. albicans has lately become a cause of great concern to the dental profession. Coexistence of Candida species, either as a commensal and/or as a pathogen, has attracted the attention of many investigators.[1] Candida albicans has been termed as a notorious opportunistic pathogen amongst the candida species. It is so termed owing to its ability to cause an infection when the host defense is either lowered or rendered inadequate. Candidiasis is a common disease among the elderly, primarily affecting the oral cavity (Oral thrush) in denture wearers. Apart from the medications available, the search for products that could help in its treatment is vital for dentists.[1,2] The global population of denture wearers is increasing, so is the incidence of denture biofilm-related problems, such as denture-associated stomatitis, aspiration

pneumonia, and malodor. It has been suggested that consumption of probiotic bacteria may improve oral health.[2,3] However, the effect of probiotics on the oral microbia of denture wearers have received little attention. In edentulous individuals, acrylic prostheses (dentures) provide a hard, nonshedding surface that facilitates fungal adhesion and subsequent colonization.[4] An essential prerequisite for candidal colonization and infection is the ability of the yeast to adhere to the superficial epithelial ce lls as well as to the fitting surface of the denture, considering the latter as a reservoir of infection. A number of materials have been tried in the fabrication of denture bases.[1,5] Among them, metals and acrylics are in vogue. In contrast to the acrylic denture bases (Polymethylmethacrylate), metallic denture bases have the advantage of presenting impervious hydrophobic surfaces and are also considered superior in many respects.[6] But, Polymethylmethacrylate (PMMA) has been widely employed in the fabrication of dentures due to its simplification and ease in the fabrication technique. Among the various denture base materials used, there may be a certain degree of variation in the factors influencing the adherence of C. albicans.[7,8] However, literature is rather scanty in reference to the adherence of C. albicans to denture base materials, so the aim of the following study is to check whether probiotics alter Candidal adherence onto the denture base material, taking PMMA into consideration in this study.

Materials and Methods:

An in vitro study was conducted to evaluate the adherence of C. albicans to PMMA denture base materials. Sample number: 10/denture base resin Sample size: 10 mm × 10 mm (length) and 2 mm (width).

Test group: PMMA samples coated with probiotics at varying concentrations dipped in saliva containing candida species.

Control group: PMMA samples in saliva containing candidal species.

Preparation of PMMA Specimens:

Test samples were fabricated in the following way using a metal die. A die was manufactured that had 10 slots, and each slot was of dimension 10 mm×10 mm and 2 mm thick. Modeling wax was taken in a glass of metal and melted on top of the flame. The melted wax was poured into the slots of the die and allowed to cool down. After the wax cooled down, removed the excess wax with a wax knife. The process was repeated again and a total of 10 wax squares were made with the help of a die. Flasking was done after the samples

were taken out of the die. Heat cure denture base resins were used at the time of packing, i.e., DPI heat cure denture base resin. The samples were fabricated by proper mixing of powder and liquid according to the manufacturer's instruction. The flasks were secured for 30 minutes in a dental clamp and polymerized in an acrylizer. A long curing cycle (70° for 9 hours) was employed to decrease the residual monomer content and also obtain good transparency in the material. They were finished and polished according to the manufacturer's instructions using standard techniques mimicking how a denture would be polished.

Saliva Samples and Probiotic coating:

After acquiring the PMMA discs of required dimensions, they were subjected to artificial aging by immersion in the saliva collected from patients with clinically diagnosed Oral Candidiasis. A sample was then collected from the disks as a baseline data for culture of candidal species.

Then the Probiotic solution (Yakult probiotic) was prepared in different concentrations (2.5, 5, 10, 15 and 20 mg/mL). Commercially available probiotic lozenges were diluted and used for this purpose. It contained Lactobacillus casei Shirota = 6.5x106, L.sporogenes =10x106 and Bifidobacterium species = 2x106 according to the manufacturer. The formed disks were dipped in the prepared probiotic solution for a period of 48 hours at 37*C. A swab sample was collected from each disk after immersion in probiotic solution as **test group** for culture of candidial species.

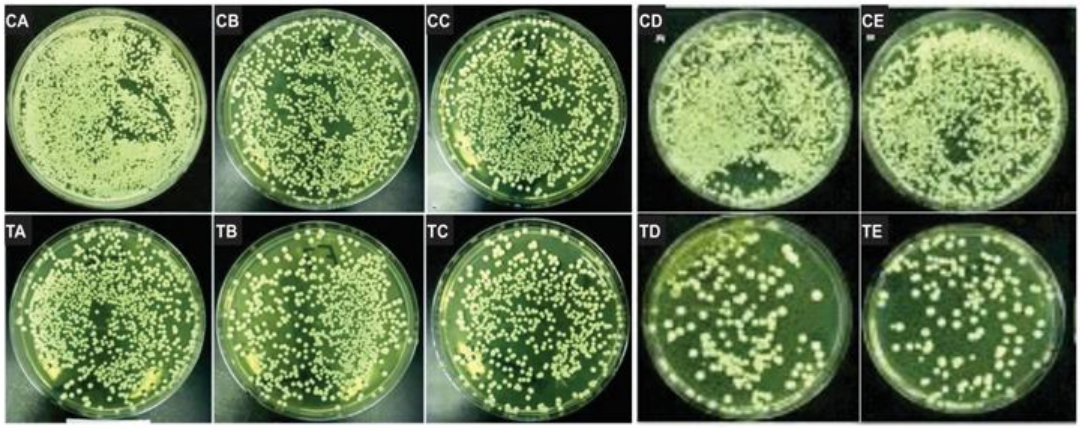
Culture preparation and Colony Counting:

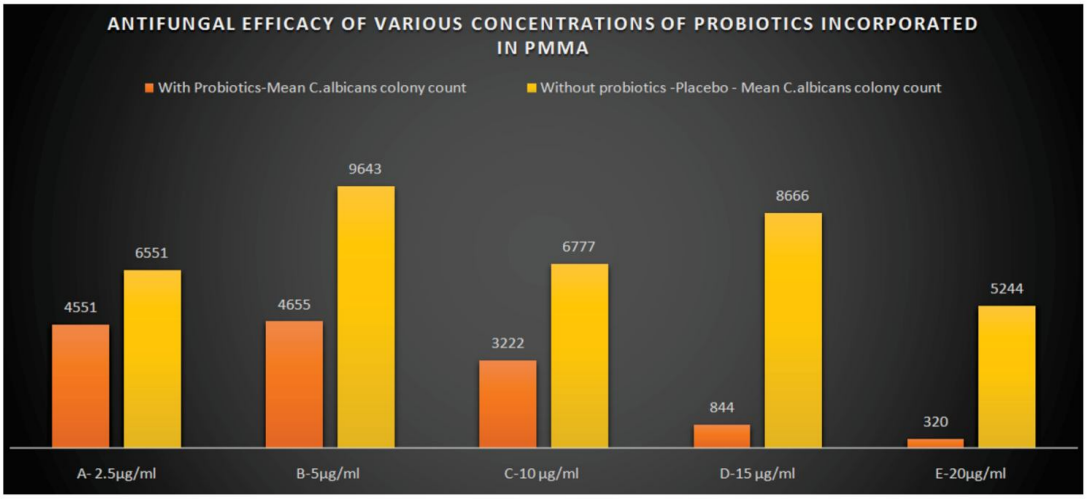
The culture preparation and the growth of C. albicans on the specimens prepared were assessed through microbial analysis. Swab samples collected from the disks before and after immersion in probiotic (Baseline and test samples) were smeared on the culture media in petri dishes and incubated for 24 hours at 37°C. This procedure is carried out inside the ultraviolet chamber. Candidal colonies were formed on the media after 24 hours. After completion of the incubation period, the specimens were removed using sterile forceps to avoid any contamination. The specimens were then fixed on a glass slide using sterile methyl alcohol for 1 minute after which the alcohol was drained. A drop of cedar-wood oil was placed over the specimen that was placed on a glass slide and observed under oil immersion lens (1000×). The same procedure was repeated as mentioned above for all the specimens and

the cells were counted microscopically. The average candidal cells on the disks before and after introducing probiotic were calculated using manual counting method and the data was tabulated and analysed using statistical analysis.

RESULTS:

We had prepared 10 samples of heat cure denture base materials and compared the adherence of Candida (Candidal colony cell count) onto them, before and after coating with probiotic solution (Fig 1). Average Candidal cell counts on heat cure denture base material before giving probiotic were compared., i.e., the difference among average Candidal cell counts on heat cure denture base materials before and after giving probiotic is significant. There was a significant decrease in Candidal cell counts with increase in probiotic concentrations on the heat cure denture base materials (Fig.2).





DISCUSSION:

Prophylaxis of Candida infection among critically ill patients is usually made with azoles or oral use of nystatin, and recent reviews concluded that both treatments have a beneficial effect in reducing Candida invasive infections. Although these local and systemic antifungals have been proven to be effective in reducing fungal colonization and invasive fungal infections, their use is not without harm.[9] Furthermore, the increased number of yeasts resistant to antifungal drugs indicates a need for new targets for new antifungal agents.[10] Few studies on probiotics against oral Candida in the oral cavity have been performed in nonasymptomatic patients. In fact, most studies focused on patients with oral candidiasis, and the probiotic was not the only treatment to reduce Candida, but rather an adjuvant of the conventional therapy. A recent study reported that the use of a probiotic product formed by Bifidobacterium longum, Lactobacillus bulgaris, and S. thermophilus in conjunction with oral local antifungal agents (nystatin) was more effective in the treatment of Candida-associated stomatitis than the conventional therapy.[11,12] The mechanisms of probiotics against oral Candida may involve a combination of factors, such as competition for adhesion sites and nutrients, production of antimicrobial compounds, stimulation of cytokine production and phagocytosis, induction of IgA secretion, maintenance of the epithelial barrier of defense, and modulation of the innate and adaptative immune response.[2,6,7]

Our particular study was done to evaluate the effect of probiotics on Candidal adherence on denture base resins. Candidal colony cell count was done on heat cure denture base materials both before and after incorporation of probiotics at varying concentrations to the samples and the results obtained were compared and used for statistical analysis.

Chhabra et al., conducted a study to evaluate the effect of denture adhesive with probiotics on CFU of Candida in completely edentulous patients. Their study results showed a decrease in the number of CFU of Candida after using denture adhesives with probiotics.[13] These results correlate with the results of our study. The present study results also correlate with the study conducted by Dos Santos et al., wherein individuals presenting with Candida in the oral cavity used probiotic Yakult LB for 20 days and showed a significant reduction in Candida prevalence (46%) and mean Candida CFU/ml counts (65%). They concluded that this reduction may be due to competition between the yeasts rather than by specific immune response stimulation.[14]

Miazima et al., through his study concluded that., Daily consumption of cheese supplemented with probiotics, with either L. acidophilus NCFM or L. rhamnosus Lr-32, was

able to reduce the colonization of oral Candida in complete denture wearers, suggesting its potential in reducing the risk of oral candidiasis in this highly susceptible population.[15] A study done by Hatakka et al., found out the effect of probiotics on the prevalence of Candida in the elderly. The study results suggested that probiotic intervention reduced the risk of high yeast and probiotic bacteria can be effective in controlling oral Candida and hyposalivation in the elderly.[6]

The data available with respect to invivo application of probiotics at varying concentration to prevent candidal adhesion to denture is still scarce and need further investigation and research.

CONCLUSION:

The study was undertaken with the objective of evaluating the effect of probiotics on Candidal adherence on heat cure denture base material. Based on the results, following conclusion can be drawn:

1. Probiotic application on denture base resin (PMMA) did decrease the candida albicans count compared to the denture base without probiotic application.
2. As the concentration of probiotic over the denture base increase, the candida cell count decreased respectively.

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