Original Article

Effect of Chitosan Nanoparticle for Controlling Fungal Biofilms on Denture Surface

S. Keerthika, P. Meenachi and R. Subashini*

Department of Biomedical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam – 603 110, Tamil Nadu, India.

Corresponding author - *Dr. R. Subashini (subashinir@ssn.edu.in)
Assistant Professor, Department of Biomedical Engineering,
Sri Sivasubramaniya Nadar College of Engineering,
Kalavakkam – 603 110, Tamil Nadu, India.

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Abstract

Denture stomatitis (DS) is a pathological disorderrelated with Candida albicans, and it is a wide spread recurring clinical complications for complete-denture wearers. The etiology and pathogenesis of Candida-associated denture stomatitis (CADS) is the initial attachment and adherence of C. albicans to the interior surface of a denture base that causes colonization and biofilm-formation which leads to DS. It is a frequent fungal infection that affects up to 67% of denture wearers. Dentures are prosthetic devices designed and formulated to substitute the missing teeth. It is supported by the adjacent soft and hard tissues of the oral cavity. The device isidealfor cases such as gum disease, tooth decay or injury. The major drawback of denture is colonization and biofilm formation over the surface of denture. The antifungal activity of Chitosan nanoparticles (ChNPs) hasbeen reported, but there is no evidence about Candida Spp biofilm on denture base surface. The main approach is to explore the effect of chitosan nanoparticle against the C.albicans biofilm on the heat cure acrylic denture disc. In the present study chitosan nanoparticle (ChNps) was synthesized and characterized using UV visible light spectroscopy. The denture disc of 13mm diameter and 1mm thickness were made and then the chitosan nanoparticle is grafted to it using dip coating method. Discs surface hardness and roughness were tested. The antifungal effect of chitosan nanoparticle and chitosan nanoparticle coated denture disc against C.albicansbiofilm adhesion and formation were tested.

Keywords: Candida albicans, chitosan nanoparticle, denture, fungal biofilm, CADS (Candida associated denture stomatitis).

Introduction

Denture stomatitis is severe clinical conditions have an harmful effect on the mucosa underside ill-fitting dentures, and the causative factor is considered as Candida albicans.[1] To treat the inflamed tissues in dentures, tissue conditioners are applied as a temporary lining material. However the antifungal activity was not exerted by the tissue conditioners and the soft surface texture harbors C. albicans easily. Along with the other clinically relevant Candida species, Candida albicansis the most common opportunistic pathogenic yeast which lives in human body.[2] Candida albicans is the common infection within the mouth of 45-65% of healthy individuals with a higher occurrence found in children and young adults. In denture wearers, the percentage of Candida infection increases from 60-100% and therefore the organism are often opportunistic; this may be lead to the decreased oxygen flow and saliva to the underlying tissue producing an acidic environment that favors the growth of yeast.

The current strategies of managing CADS, includes enhancing the habits of denture wearer, cleaning and sterilizing, applying tissue conditioners or soft liners, and systemic antifungal treatments. However, the above said methods cannot completely eradicate the Candida colonization and biofilm formation. Therefore, the risks of CADS are high, mainly among immunity compromised patients. An alternative approach is to incorporating denture materials with antifungal drugs for localized delivery at the site of infections.[4-8]

The unique properties such as bioactivity, antimicrobial, biocompatibility and compatibility to blend with other materials is considered, chitosan as a potential material for dental applications.[9-11] Nystatin, Metronidazole, chlorhexidine which is used as a prophylactic agent for oral mucositis was integrated into the formulations.Chitosan in the form of nanoparticle and resorbable film used for controlled drug delivery ofnystatin, metronidazole and chlorhexidine to periodontal tissues in situ,against fungal infections and oral mucositis.[12-13]

This study investigates the antifungal effect of the chitosan nanoparticle prepared using ion gelation method against the biofilm formation of candida albicans. Agarwell diffusion method was used for antifungal test with different concentration of chitosan nanoparticles and coated denture surface.

Methodology

A. Materials

All the chemicals used in the present study were of analytical grade and procured from Fischer Scientific Company, Waltham, MA. Candida albicans (ATCC10231) was received from the Life Tech Research Centre.

B. Preparation of Chitosan Nanoparticles

Chitosan nanoparticleswere prepared using Ion gelation method.[14-15] Low molecular weight (LMW; MW=107 kDa) chitosan powder with 75–85% degree of deacetylation was used. Since chitosan does not soluble in water, acetic acid is used to dissolve. Chitosan in the concentration of 5.0 mg/mL was mixed with 1% v/v acetic acid solution. The solution was kept under magnetic stirring for 24h until clear solution is obtained. The TPP solution was prepared in the concentration of 2.5 mg/mL and mixed with 10 mL of chitosan solution using dropper. The prepared solution was kept under constant stirring at 800-900 rpm for 30mins. Samples were visually observed and categorized into three different categories via: clear solution, opalescent suspension, and aggregates.

Then, the prepared solution is tested to confirm the formation of nanoparticles using UV-Vis spectroscopy.[15] To verify the formation of nano- particles the solution was scanned in the range of 200–600 nm in a spectrophotometer using a quartz curette with water as the reference. The obtained data from the uv-vis spectrometer is fed into the Origin pro 2016 software to plot the graph. The absorption peaks for ChNPs were obtained at 300nm.

C. Denture Disc

DPI heat cure acrylic resin was used to create the denture disc. Acrylic resin is a resinous plastic material used as a denture base material. The formulation of denture base material includes Powder: polymethyl methacrylate PMMA (polymer) + Benzoyl peroxide (initiator) + pigments and Liquid: methyl methacrylate (monomer) + hydroquinone (inhibitor). Powder and liquid are mixed in a ratio of 3:1 for fabricating average sized denture material. The preparation is a two-componentresin, comprised of PMMA and methylmethacrylate (MMA) with the addition of ethylene glycol dimethacrylate (EGDMA) as the cross linking agent. The two components were mixed through heat-polymerization (90 min at 73°C and then 30 min at 100°C) and the denture resin discs were fabricated The resultant discs are 13mm diameter and 3mm thickness. Fifteen heat cure acrylic resin disc were made by heat polymerization. Each disc is of 13mm diameter and 3mm thickness.

D. Grafting Chitosan Nanoparticle on Denture Disc

The prepared chitosan nanoparticle is grafted to the denture discs using the spin coating method. Spin coating is the process where the substance is deposited on the surface by spinning speed. The chitosan nanoparticles is placed in the centre of the disc and then the 1000rpm(rotation per minute) was set for 2seconds and then the disc is air dried and the procedure repeated for the other side of the disc respectively. Then the thin film of chitosan nanoparticle is formed on the disc. Further, the hardness and roughness of the disc after coating were tested.

E. Spin Coating

The sample (Denture disc) was spinned at high speed (>10 rotations per second = 600 rpm) and the centrifugal force fused with the surface tension of the solution pulls the liquid into anuniformcoating. During this process the solvent evaporates to leave the prepared chitosan nanoparticles on the substrate in ahomogenous deposition (Fig 3).

F. Surface roughness and Surface Hardness

The denture surface sample was placed under the surface roughness-measuring instrument with the cutoff value set at 0.08mm (Gauss filter). It is equippedwith 5 µm radius diamond needle, which traversed the surface at a continual speed of 0.05 mm/s with a force of 4 mN. The micro hardness measurements were performed using a Vickers indenter (HV) and set the load of 50 g with a time interval of 10s. Five indentations were done for each specimen, at least 100 µm apart and the mean value of HV was measured. The surface roughness and hardness were tested after coating ChNps on denture surface and the values are represented in Table 2.

G. Antifungal study

The fungal culture was incubated and maintained on potato dextrose agar (PDA) medium at 28°C. The fungal inoculums candida albicans was prepared from 5-10 days old grown on Potato dextrose agar medium.

Well diffusion method was used to detect the antifungal effect against candida albicans.16 The potato dextrose agar plate was inoculated with the 10 days old fungal culture using point inoculation. The 5mm in diameter filter paper was impregnated with different composition of prepared chitosan nanoparticles(10µl, 20µl, 30µl). ChNPS (10µl, 20µl,

30µl) coated denture surface was also tested for its antifungal activity. Chloramphenicol was used as positive control. Zone of inhibition was measured after theincubation period of 72 hrs at 28°C.

Results and Discussion

In present study, antifungal effect of the candida albicans was tested. Candida albicans was the main cause of fungal infection in denture wearers. Chitosan is the natural polymer that is obtained from the exoskeleton marine animals (such as lobster, shrimp, etc).17-18 Chitosan nanoparticles have the antimicrobial property. The chitosan nanoparticle

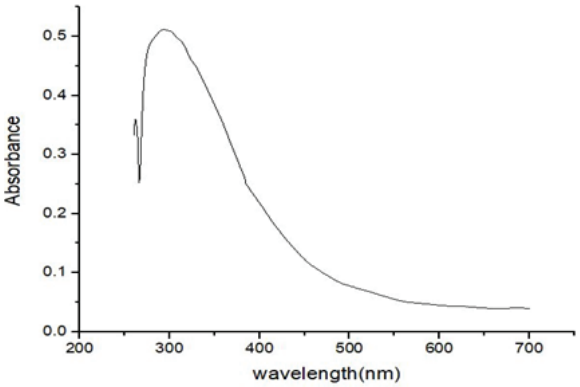

Figure 1: UV-vis spectrum of Chitosan nanoparticle

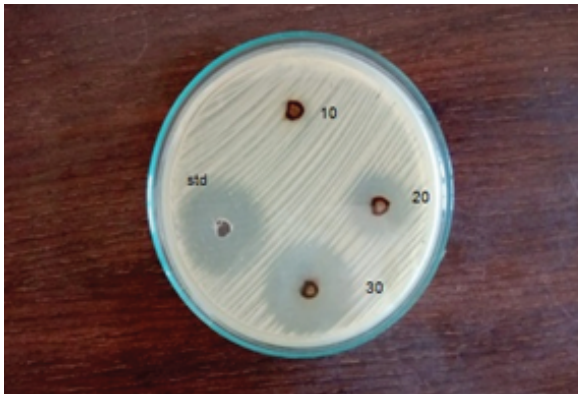

Figure 2: Antifungal study of ChNPS

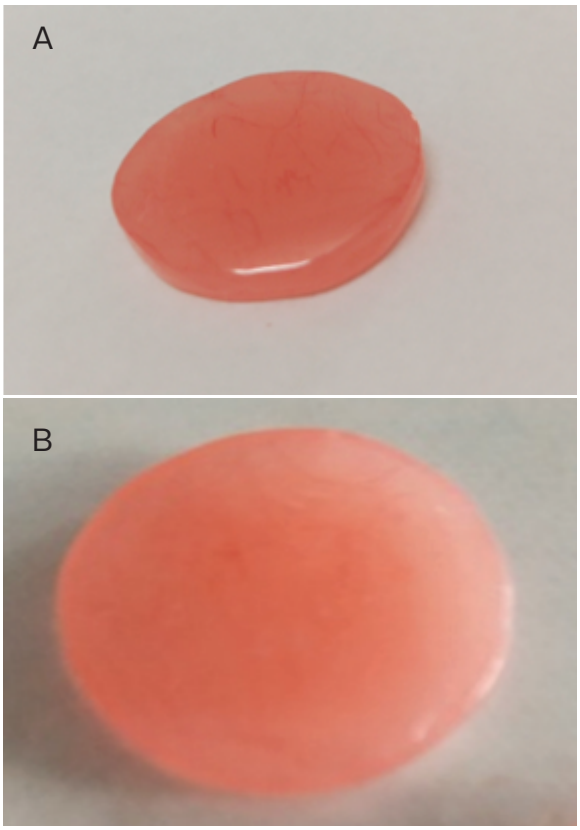

Figure 3: (A) Denture disc (B) Denture disc coated with chitosan nanoparticle

was prepared and characterized using UV-Visible spectroscopy. In this water is kept as the reference sample and characterized. The uv-vis spectroscopy shows the peak at 300nm wavelength. This indicates the formation of chitosan nanoparticle (Fig. 1).

The antifungal property of the synthesized chitosan nanoparticle was tested against the biofilm formation candida albicans.19-21 The candida albicans was grown on the potato dextrose agar. The antifungal property was tested using well diffusion method for different concentration of chitosan nanoparticle solution (10µl, 20µl, 30µl) and the standard solution. The standard solution used is Chloramphenicol (10 µl).The experiment was conducted in triplicates

Sl. No.	Sample	Zone of inhibition (mm)			Standard (Chloramphenicol)
		10 µl	20 µl	30 µl	10 µl
1.	ChNPS	00±0.00	04 ± 0.15	08 ± 0.15	09 ± 0.20
2.	ChNPS coated Denture surface	02±0.11	06 ± 0.14	11 ± 0.32	

Table 1: Zone of inhibition of Candida albicans for different concentration of Chitosan nanoparticle

Surface roughness (µm)	Microharness (Hv)
0.042 ± 0.006	20.3 ± 0.62

Table 2: Roughness and hardness of ChNPS coated Denture surface

(n=3). The 30 µl has shown the maximum zone of inhibition than the other two. Figure 2 shows the antifungal effect of chitosan nanoparticle against the biofilm formation of candida albicans. The zone of inhibition was measured in millimeter using vernier caliper and the values are shown in Fig 2 and table 1.

Conclusion

Drug delivery is the better approach to completely prevent the biofilm formation of candida species on the denture surface.Chitosan nanoparticle have antifungal property against the candia albi-can, which shown the clear inhibition zone in the result. Chitosan nanoparticle has been synthesized using sodium tripolyphophate and acetic acid solu-tion. The characterization of the chitosan nanoparticle was done using UV-vis spectroscopy and the obtained peak of 300nm shows the formation of chitosan nanoparticles. Then the antifungal effect of chitosan nanoparticle against candida albicans was tested using well diffusion method.The results obtained shows that chitosan nanoparticle have antifungal property to inhibit the growth of candidaalbicans.

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