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Antifungal efficacy of tissue conditioner incorporated with nano graphene oxide: an in-vitro study

[1]Dr.Sounyala.Rayannavar, Reader, Department of prosthodontics and crown and bridge, KLE Academy of higher education and research's Vishwanath Katti Institute of dental sciences, Belagavi, Karnataka, India.

[2]Dr.Bhumika.Suresh.Shaha, Post Graduate student, Department of prosthodontics and crown and bridge, KLE Academy of higher education and research's Vishwanath Katti Institute of dental sciences, Belagavi, Karnataka, India.

[3]Dr.Swapnil.Shankargouda, Senior Lecturer, Department of prosthodontics and crown and bridge, KLE Academy of higher education and research's Vishwanath Katti Institute of dental sciences, Belagavi, Karnataka, India.

[4]Dr.Santosh Nelogi, Reader, Department of prosthodontics and crown and bridge, KLE Academy of higher education and research's Vishwanath Katti Institute of dental sciences, Belagavi, Karnataka, India.

Corresponding author: Dr. Swapnil Shankargouda, Senior Lecturer, Department of prosthodontics and crown and bridge, KLE Academy of higher education and research's Vishwanath Katti Institute of dental sciences, Belagavi, Karnataka, India.

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Abstract

Purpose: Candida induced stomatitis is of main concern for most of the old denture wearer patients. Combination of tissue conditioner with antifungal agent can be an effective solution to enhance the compromised tissue health. The aim of the study was to evaluate and compare the antifungal efficacy of tissue conditioners when incorporated with different concentrations of nano graphene oxide against Candida albicans.

Materials and methods: Nano graphene oxide was added in 1%, 3% and 5% by weight to the acrylic-based tissue conditioner (GC Soft liner) to form three study groups A, B and C respectively. A total of five plates were prepared using Sabouraud dextrose agar (SDA) and inoculated with 24-hour old *Candida* culture. Antifungal susceptibility was checked with well diffusion method. The acrylic-based tissue conditioner (GC Soft liner)-nano graphene oxide concentrations were mixed according to the groups and placed into the wells. The diameter of inhibition zone was measured after end of 1 day (24 hours) and 7 days.

The obtained data was subjected to statistical analysis using one-way ANOVA, Tukey's multiple post hoc test and Dependent t test.

Results: Addition of nano graphene oxide in tissue conditioner showed statistically significant increase in the antifungal activity against Candida albicans. The acrylic-based tissue conditioner (GC Soft liner) with 5% nano graphene oxide showed highest diameter of inhibition zone followed by addition of 3% nano graphene oxide and least with 1% nano graphene oxide concentration both after 1 day and 7 days.

Conclusion: Tissue conditioner combined with nano graphene oxide is effective against Candida albicans. Increasing the concentration of nano graphene oxide increases the antifungal activity of the acrylic-based tissue conditioner (GC Soft liner).

Keywords: Soft liner, Candida albicans, antifungal activity, denture stomatitis.

Introduction

Denture wearers often face the problem of tissue inflammation due to neglected oral hygiene and poor maintenance of the dentures leading to food accumulation and subsequent plaque formation.[1] This inflammatory condition is known as denture stomatitis which affects around 50% of patients wearing maxillary complete denture.[2]

Candida albicans is the opportunistic microbe predominantly involved in denture stomatitis. In healthy individuals 40% to 60% of microbial flora is comprised of Candida albicans and causes human candidiasis in 50% to 90% of infections.[3]

Tissue conditioners are used to reline the dentures with traumatized denture bearing tissues. They can be applied for few days to weeks depending on the improvement of tissue health.[2] Denture base and soft lining material enhances growth of Candida albicans if hygiene is not maintained properly. Surface properties of tissue conditioner, pH and composition of saliva, carbohydrate rich diet and compromised immunity are some factors which are responsible for increased susceptibility to infection.[3]

To overcome this problem, it is necessary to incorporate tissue conditioners with antifungal agents. Soft liners with antifungal medicaments like nystatin, fluconazole, itraconazole, chlorhexidine have shown to be effective against Candida albicans.[1,4]However, prolonged use of these medicaments may lead to development of resistant fungal strains.[5] Recent studies have proved antifungal efficacy of various nano materials such as magnesium oxide (MgO), zinc oxide (ZnO), silver nanoparticles when mixed with tissue conditioners.[1,2,6]

Graphene is a two-dimensional carbon allotrope with single layer sp^2 bonded carbon atoms in hexagonal configuration. Studies have shown the potential application of graphene and its derivates in restorative dentistry as filler material, bone tissue engineering, coating for dental implants.[7]Graphene oxide is a single or few layered graphene formed by oxidizing the graphite. Nano graphene oxide (nGO) is known to have antimicrobial activity through surface contact. Sharp nanosheets causes perturbation of cell membrane, electrolyte leakage and eventually cell death.[8]

Considering this, the present study aims to evaluate and compare the antifungal efficacy of tissue conditioner incorporated with graphene oxide against *Candida albicans*. Null hypothesis states that there is no difference in antifungal efficacy when different concentration of nGO is used.

Materials and Methodology

Preparation of nGO-incorporated tissue conditioner

Nano graphene oxide (nGO) powder (Graphene Supermarket, NY, USA) and acrylic-based tissue conditioner (GC Soft liner, GC Corporation, Tokyo, Japan) was used in the study. nGO powder was added in 1%, 3% and 5% by weight relative to the acrylic-based

tissue conditioner (GC Soft liner) powder and thoroughly mixed. Thus, three groups were considered in the study as group A (GC Soft liner + 1% nGO), group B (GC Soft liner + 3% nGO) and group C (GC Soft liner + 5% nGO).

Antifungal activity

Candida albicans (American type culture collection catalogue number 90028 strains) was obtained and sub cultured on Sabouraud dextrose agar (SDA) tube and incubated at 37˚C. The Candida albicans suspension after 24 hrs of incubation was then mixed with sterile saline to a density of 0.5 McFarland to standardize the concentration. Antifungal susceptibility was checked with well diffusion method using SDA on 90-mm diameter petri plates. The SDA plates were streaked with inoculum of Candida albicans using inoculation loop. After drying of inoculum, 6mm diameter and 5mm depth wells were punched with a sterile cork borer.

Tissue conditioner liquid and powder with 1%, 3% and 5% nGO concentration was mixed according to manufacturer's instructions and filled in the punched-out wells. A total of five plates were prepared and were incubated at 37ºC for 7 days. The diameter of inhibition zone (DIZ) was measured using metallic scale after end of 1 day (Figure 1) and 7 days (Figure 2).

Figure 1: Showing diameter of inhibition zone after 1 day.

Figure 2: Showing diameter of inhibition zone after 7 days.

The obtained data was subjected to statistical analysis using one-way ANOVA, Tukey's multiple post hoc test and Dependent t test.

Results & Discussion

The mean DIZ for group A, B, C after 1 day was 8.4mm, 12.8mm, 17.2mm respectively and mean value decreases after 7 days to 7.4mm, 10.4mm and 14.4mm respectively. Thus, The acrylic-based tissue conditioner (GC Soft liner) with 5% nGO showed highest antifungal activity followed by addition of 3% nGO and least with 1% nGO concentration both after 1 day and 7 days.

Table 1 represents one-way ANOVA test shows that statistically significant difference ($p<0.05$) was seen for mean DIZ between the three study groups after 1 day, 7 days and difference in 1-7 days. Thus, addition of different concentration of nGO in tissue conditioner had significantly increased the antifungal activity against Candida albicans.

Tukey's multiple post hoc test reveals that statistically significant difference ($p=0.001$) was seen for inhibition zone between each pair of group after 1 day, 7 days and difference in 1-7 days, except for group B (3% nGO) and

group C (5% nGO) for the difference in 1-7 days which was insignificant (p=0.056) as shown in Table 2.

Dependent t test to compare inhibitory efficiency after 1 day and 7 days shows statistically significant difference for each group with values for group A (p=0.034), group B (p<0.001) and group C (p<0.001). Addition of nGO in the acrylic-based tissue conditioner (GC Soft liner) in 1%, 3% and 5% concentration had significant antifungal activity even after 7 days as shown in Table 3.

Treatment	Sources of variation	Degrees of freedom	Sum of square	Mean sum of square	F-value	p-value *p<0.05
After 1 day	Between groups	2	193.6	96.8	170.8235	<0.001*
	Within groups	12	6.8	0.56		
	Total	14	200.4			
After 7 days	Between groups	2	123.33	61.66	132.1428	<0.001*
	Within groups	12	5.6	0.46		
	Total	14	128.93			
Difference 1-7 days	Between groups	2	8.4	4.2	31.5	<0.001*
	Within groups	12	1.6	0.13		
	Total	14	10			

Table 1 shows the statistical comparison of mean diameter of inhibition zone of three study groups after 1 day and after 7 days by one-way ANOVA

Time	Groups	Mean difference	P-value *p<0.05
After 1 day	A vs B	4.4	0.001*
	A vs C	8.8	0.001*
	B vs C	4.4	0.001*
After 7 days	A vs B	3	0.001*
	A vs C	7	0.001*
	B vs C	4	0.001*
Difference 1-7 days	A vs B	1.2	0.001*
	A vs C	1.8	0.001*
	B vs C	0.6	0.056

Table 2 shows pair wise comparisons of mean diameter of inhibition zone of three study groups after 1 day and after 7 days by Tukeys multiple post hoc procedures

Groups	Treatment	Mean	S.D.	Mean Diff.	Paired t	P-value *p<0.05
A	After 1 day	8.4	0.89			
	After 7 days	7.4	0.55	1.0	-3.1623	0.034*
B	After 1 day	12.8	0.84			
	After 7 days	10.4	0.55	2.4	-9.7980	<0.001*
C	After 1 day	17.2	0.84			
	After 7 days	14.4	0.89	2.8	-14	<0.001*

Table 3 shows the comparisons of mean diameter of inhibition zone after 1 day and after 7 days in the three study groups by dependent t test

Denture stomatitis is most frequently seen in old denture wearers and is multifactorial in origin. It is caused by tissue trauma from ill-fitting dentures, poorly maintained denture hygiene, dietary factors, xerostomia, continuous denture use without removal and compromised medical condition.[4]

Candida infection is strongly associated with denture stomatitis with Candida albicans being the primary microbe. Microbial adhesion on denture base material is based on substrate characteristics, physical/chemical properties and denture cleaning measures.[9]

Tissue conditioners are used to treat traumatized inflamed tissue by relining the dentures for temporary period. They improve the retention of the prosthesis and also provide the cushioning effect.[6] However; microorganisms can easily adhere to the tissue conditioners due to their soft texture making it difficult for mechanical cleansing. Okita et al (1991) proved that microbial colonization was more with tissue conditioners as compared to acrylic resin.[10]

Thus, to reduce the microbial colonization it is necessary to incorporate antibacterial and antifungal agents to these materials to improve the tissue health. This study aimed to

evaluate antifungal efficacy of tissue conditioner when incorporated with different concentrations of nGO.

Graphene is one of the known antimicrobial agents. Di Giulio M et al (2018) have proved that graphene oxide shows antimicrobial and antibiofilm efficacy against chronic wound microorganisms including Candida albicans.[11] Its blade like configuration physically damages the microbial cellular membrane and causes leakage of intercellular substance leading to cell death. Wrapping and photothermal ablation mechanism also lead to cell damage. Chemical production of ROS also causes disintegration of cell membrane and eventual cell death.[12]

The acrylic-based tissue conditioner (GC Soft liner) alone does not show any antifungal activity.[2] Minimum inhibitory concentration (MIC) of graphene oxide against nosocomial pathogens is proved to be in the range of 2.5 to 5.[8] Thus, current study compared three different concentration of nGO: 1%, 3% and 5% by weight with respect to the acrylic-based tissue conditioner (GC Soft liner).

Present study showed that DIZ was maximum with addition of 5% nGO for both after 1 day and 7 days with mean values of 17.2mm and 14.4mm respectively. Minimum DIZ was seen with addition of 1% nGO for both after 1 day and 7 days with mean values of 8.4mm and 7.4mm respectively. Study done by Kanathila H et al (2011) showed similar results with DIZ for Candida albicans between 6mm-21mm when magnesium oxide was added in different concentration to soft liners.[2]

Lee JH et al (2018) proved that 2% nGO incorporated in polymethyl methacrylate (PMMA) showed sustained antimicrobial adhesive property against Candida albicans for up to 28 days.[13] Gamal R et al (2019) showed that 0.05% nGO addition to PMMA inhibits the growth of Streptococcus mutans.[14]

However, present study showed enhanced antifungal activity by increasing the concentration up to 5%. This may be due to difference in the chemical composition of PMMA and soft liner and methodologies used due to different strains.

Graphene oxide has also enhanced the antimicrobial activity when used in combination with other materials/drugs. Kulshrestha S et al (2014) showed that graphene/ zinc oxide nanocomposite inhibited Streptococcus mutans efficiently and can be used as coating agents for dental implants.[15] Shahi SA et al (2019) found out antifungal activity of graphene oxide in combination with fluconazole against Candida albicans through DNA fragmentation with low cytotoxic effect.[16]

Clinical significance

The current study rejects the null hypothesis. Increasing the concentration of nGO showed significantly increased antifungal activity. Thus, nGO can be used to reduce the fungal growth and has potential to treat candida induced denture stomatitis by drug delivery through tissue conditioners.

Limitations and future scope

One of the shortcomings of the study was that addition of nGO gave blackish discolouration to the soft liner. To overcome this, future studies can concentrate to coat the graphene material to mask the colour before incorporating into substrate. This in vitro study included observation till 7 days. In many treatment modalities use of soft liner can extend up to several weeks. Therefore, along with the cytotoxicity test, the durability of sustained drug delivery should be evaluated for further clinical use. Future studies can be planned investigating the effects on substrate's mechanical properties after incorporating nGO and to find out least concentration required to provide potential antifungal activity without altering the physical properties.

Conclusion

Within the limitations of this study, following conclusions can be drawn:

- Significant increase in the antifungal activity against Candida albicans was seen when nGO was incorporated in the acrylic-based tissue conditioner (GC Soft liner).
- Increasing the concentration upto 5% nGO showed maximum DIZ after 1 day and 7 days with the mean values of 17.2mm and 14.4mm respectively.

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