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Research Article

A STUDY TO EVALUATE THE ANTIFUNGAL EFFICACY OF NANOGRAPHENE OXIDE INCORPORATED IN MAXILLOFACIAL PROSTHESIS MATERIAL –AN INVITRO STUDY

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Introduction: Maxillofacial prosthesis made of medical grade silicone elastomers are routinely used to replace facial parts lost through diseases or trauma. The prosthesis needs to be biocompatible to enable wound healing and the restoration of healthy tissue; but the material also needs to be aesthetically acceptable to the patient. Hence this basic cell viability analysis was planned to evaluate antifungal efficacy of nanographene oxide incorporated in maxillofacial prosthesis material.

Material and methods: Thirty wax samples of 6mm diameter and 1mm in thickness were made in a denture acrylisation flask. This flask was heated in the boiling water to dewaxing procedure, which removed the wax patterns from these 30 moulds

Results: Silicon discs incorporated with graphene oxide showed antifungal activity against candida albicans whereas silicon discs that is the control group showed no activity.

Conclusion: Incorporation of Graphene oxide in the maxillofacial silicone material can reduce the fungal growth.

INTRODUCTION

Maxillofacial prosthesis made of medical grade silicone elastomers are routinely used to replace facial parts lost through diseases or trauma. The prosthesis needs to be biocompatible to enable wound healing and the restoration of healthy tissue; but the material also needs to be aesthetically acceptable to the patient. (Aziz T *et al*, 2003), (Hooper SM *et al*, 2005).

Maxillofacial prostheses are exposed to saliva and nasal secretions, (Kurtulmus H *et al*, 2010) and thus they are inevitably susceptible to bacterial colonization, which usually leads to the subsequent degradation of the material and infection of the surrounding tissues. (Busscher HJ *et al*, 1994). There is a wide range of microbial species that are known to colonize the biomaterials used for prostheses. (Campoccia D *et al*, 2013).

The occurrence and severity of fungal infections have significantly increased in recent decades, with high rates of morbidity and mortality, especially in immunocompromised patients. (Chang Y *et al*, 2017), Vazquez JA (2007). It is estimated that fungal diseases affect approximately 1.2 billion individuals worldwide with at least 1.5 million deaths each year.(Chang Y *et al*, 2017), (Sandhu R *et al*, 2017).

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Candidiasis is an important opportunistic fungal infection, causing the major morbidity and mortality in immunocompromised patients with underlying disorder such as neutropenia, post chemotherapeutic state, malignancies, indwelling catheter, maxillofacial prosthesis, subjects undergoing corticosteroid therapy and consuming broad-spectrum antimicrobial agents. (Sandhu R *et al*, 2017), (Bassetti M *et al*, 2017). On the other hand, the growing incidence of drug -resistant candida strains and the challenge for the treatment of recurrent candidiasis because of adverse drug interaction and prescription high doses of common drugs is reported recently. Nowadays applying the safe and natural components with antimicrobial activity is highly interested. Indolicidin (IN) is a member of the cathelicidin family and one of the smallest natural linear antimicrobial peptides (AMPs) isolated from the granules of bovine neutrophils.(Pappas PG *et al*, 2015), (Sanguinetti M *et al*, 2015). Previous studies indicated the antimicrobial effect of IN against gram-negative and gram-positive bacteria, fungi, and protozoa. Because of some unique properties, including the short length and wide range of antimicrobial activity, it can be considered as an applicable candidate for the improvement of antimicrobial drugs.(Aley SB *et al*,1994),(Selsted ME *et al*, 1992), (Rozek A *et al*, 2003), (Ryge T *et al*, 2004).

Graphene oxide (GO) is a nanomaterial with two-dimensional monolayer sheet of carbon atoms and plays an important role

in drug delivery, tissue engineering, antibacterial, anticancer therapy and bioimaging. (Wu S-Y *et al*, 2015). Previous studies express the antimicrobial activity of graphene oxide (GO) against fungal and bacterial pathogens.

The benefits of GO include convenient cellular uptake and frequent hydrophilic groups on the surface. However, the biological application of Go in maxillofacial prosthesis is not known. Hence this basic cell viability analysis was planned to evaluate antifungal efficacy of nanographene oxide incorporated in maxillofacial prosthesis material.

MATERIALS AND METHODS

Thirty wax samples of 6mm diameter and 1mm in thickness were made in a denture acrylisation flask. This flask was heated in the boiling water to dewaxing procedure, which removed the wax patterns from these 30 moulds. Thirty moulds were made,out of which in 15 moulds silicone mixed with 5% of nano graphene oxide that is 50mg of graphene oxide in 1g of silicone material was mixed into a homogeneous paste by stirring thoroughly on a glass slab and filled in the mould, this was considered as study group (Group 1). The other 15 moulds were filled with a plain silicone mix without incorporating graphene oxide, which was the control group (Group 2) (Fig 1). The mixed paste was packed into the preformed disc moulds and allowed to cure for 24 hrs. at room temperature. The discs are retrieved after 24 hrs. and stored in closed container. Out of the 30 in total discs obtained from after curing, 15 black silicone discs (colored changed due to incorporation of the graphene) were considered study group discs, and other 15 discs which were control group obtained from just the mix of the silicone were considered as the control group samples.

Candida albicans (MTCC 183) was obtained and sub-cultured on Sabouraud dextrose agar (SDA) tube and incubated at 37℃. 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 inoculums of Candida albicans. Then the plates were incubated and zone of inhibition was measured using Vernier calliper. Anti-adherence test was done by growing samples on Sabouraud dextrose broth culture overnight and incubated at 37°c. after which the samples were washed with PBS solutions to remove the loosely attached microbes, followed by wash with 0.9% saline to remove the adhered cells. Samples were then subjected to spectrophotometer and absorbance at 530 nm and plated on to rose Bengal agar with chloremphenicol. Samples were incubated for 24 -48 hrs before the colony count was determined.

Stastistical analysis

According to G power software - sample size is 6, 3 in each group, considering α error as 1% and power of the study as 90 % and effect size (d) is 8.92 (according to the reference article 1) the sample size came out to be 6 i.e. 3 in each group, but for better results and statistical analysis we are taking sample size as 30 i.e. 15 in each group. The obtained data will be subjected to students- t test statistical analysis and the result will be tabulated.

RESULTS

Silicon discs incorporated with graphene oxide showed antifungal activity against candida albicans whereas silicon discs that is the control group showed no activity (Fig 2) (Table 1). After that the Reactive Oxygen species generation (ROS) of GO nanosheets was able to chemically react with the organic functional groups of chitin and other polysaccharides on the cell walls of fungi and induce antifungal activity (Fig 3) (Table 2). Also more number of cell colonies were seen with the silicon discs as compared to graphene incorporated discs.

Table 1 Values of the inhibition assay

Sample No	Zone of inhibition in diameter [mm]	
	Silicone sample	5% Granphene sample
1	-	12
2	-	11.8
3	-	12.1
4	-	11.6
5	-	11
6	-	11
7	-	10.9
8	-	11
9	-	11.2
10	-	11.2
11	-	11.8
12	-	11.2
13	-	12
14	-	11.8
15	-	11.7

Table 2 Table showing the colony count

Sr.no	Graphine sample	Absorbance (530nm)	Colony count
1	G1	0.040	2.0×10^5
2	G2	0.044	1.5×10^5
3	G3	0.036	1.1×10^5
4	G4	0.042	1.9×10^4
5	G5	0.041	2.3×10^4
6	G6	0.038	2.1×10^4
7	G7	0.039	2.4×10^4
8	G8	0.042	1.4×10^4
9	G9	0.043	1.3×10^4
10	G10	0.035	1.6×10^4
11	G11	0.037	1.4×10^4
12	G12	0.040	1.9×10^4
13	G13	0.041	1.6×10^4
14	G14	0.039	1.3×10^4
15	G15	0.033	1.1×10^4

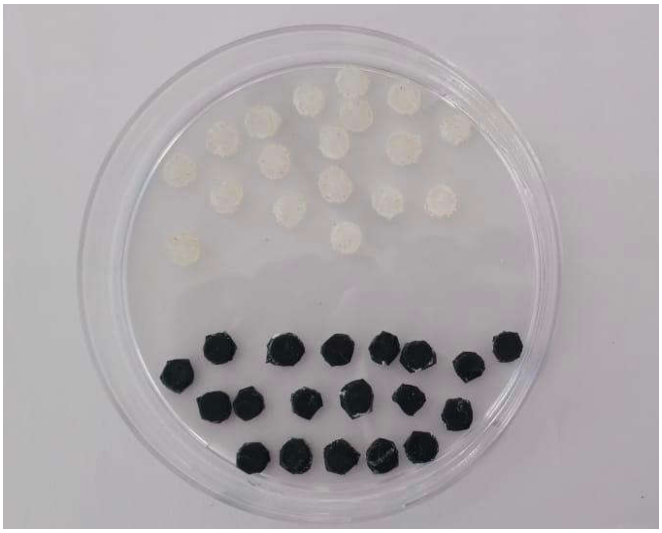

Fig 1 Prepared samples, both control and test samples

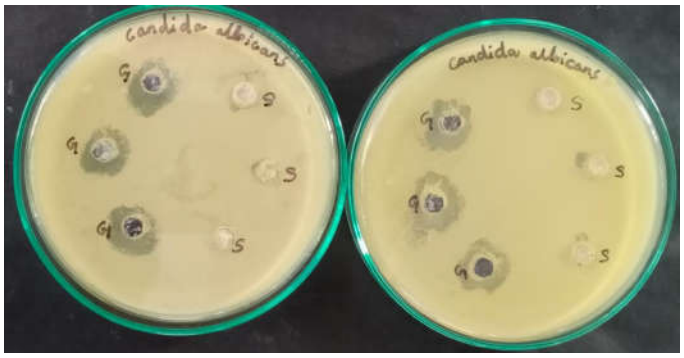

Fig 2 Zone of Inhibition assay

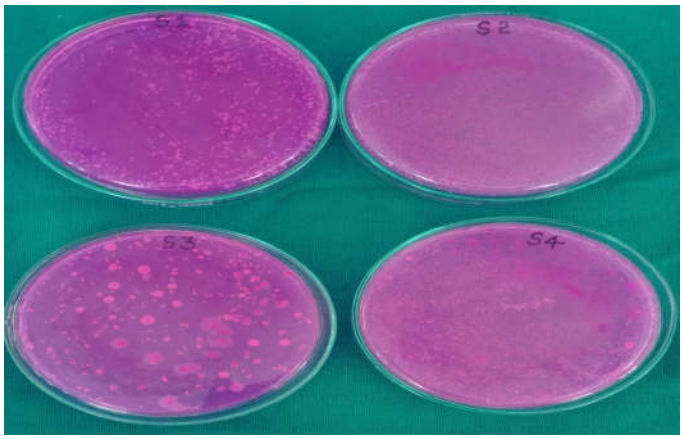

Fig 3 Showing the culture growth in anti- adhesive assay

DISCUSSION

The occurrence and severity of fungal infections have significantly increased in recent decades, with high rates of morbidity and mortality, especially in immunocompromised patients. (Chang Y *et al*, 2017), Vazquez JA (2008). Furthermore, the widespread use of antifungal drugs are bringing serious consequences for antifungal therapy, due to the emergence of resistant strains and therefore leading to treatment failures. Vazquez JA (2008), Campoys (2007).

Candidiasis is considered as an important opportunistic fungal infection as mentioned above, causing the major morbidity and mortality in immunocompromised patients. On the other hand, the growing incidence of drug-resistant Candida strains and the challenge for the treatment of recurrent candidiasis because of adverse drug interaction and prescription high doses of common drugs is reported recently. The present study which was designed to evaluate antifungal efficacy of nanographene oxide incorporated in maxillofacial prosthesis material.

Graphene oxide is the product of oxidation of graphene; therefore, it is a complex mixture of materials. Hence, due to lack of analytical techniques to precisely characterize this amorphous material with berthollide character (i.e., nonstoichiometric atomic composition), presenting an unambiguous model for GO structure is still subject of considerable debate. Despite these obstacles, different researchers have proposed different models. Despite the primary developed models that are usually based on the consideration of lattice-based model, new models have focused on an amorphous and nonstoichiometric alternative.(Nakajima T *et al*,1988), (Lerf *et al*,1998).

Cytotoxicity effect of nanocomposite and fluconazole was assessed using MTT assay. The result showed that the silicon discs incorporated in the graphene oxide antifungal gel reduction in the fungal growth. The probable antifungal mechanism of nano graphene was because of the interaction of GO nanosheets with the cell walls of fungi.

Graphene that are aggregated in suspension can isolate the bacteria from the nutrient-rich surrounding environment, (Pham VT *et al*, 2015) while nanosheets of GO can be functionalized easily and disperse water excellently. Although these are beneficial qualities of GO, the antibacterial activity of GO nanosheets is highly dependent on their size. Larger GO nanosheets express stronger antibacterial activity against E. coli, and GO itself decreases the viability of dental pathogens depending on the concentration of GO present. (Lui S *et al*, 2012), (HeJ *et al*, 2015). Graphene and some of its composites can also act against bacterial biofilms in addition to single bacteria. Karatan PJM (2009). Relating to the previously mentioned antibacterial ability of graphene against Gram-positive and Gram-negative bacteria, this is dose-dependent as well. While high GO concentrations inhibit the formation of Gram-positive and Gram-negative bacteria biofilms, low GO concentrations can actually enhance their formation, creating a completely adverse reaction to the intended. (Liu S *et al*, 2012). Although it may seem that graphene it self has an antibacterial effect on bacteria itself and bacterial biofilms, it has been proven that less than 50 μg/mL GO in a nutrient medium solution has no antimicrobial activity and actually enhances bacterial growth by acting as a biofilm itself. However, GO-polyoxyalkyleneamine, which is two antibacterial molecules in the same concentration, exhibits antibacterial activity where bacteria are grown in phosphate buffered saline solution. (Wu P-c *et al*, 2018)

CONCLUSION

Within the limitation of the study, it can be concluded that

1. Incorporation of Graphene oxide in the maxillofacial silicone material can reduce the fungal growth.
2. More number of cell colonies were seen with the silicon discs control group as compared to graphene incorporated discs (study group).

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