Antimicrobial Efficacy of Nano Paint for Die Stone Dental Cast Disinfection

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Abstract
Aim: The aim of this study is to evaluate and analyse the antimicrobial activity of nanopaint immersed die stone, where the nanoparticle used is silver nanoparticle incorporated with green tea and neem. Materials and methods: First, a concentrated solution of neem was prepared using neem extract powder. Similarly, in the same concentration, a green tea solution was also prepared in another beaker. Both of these were filtered, mixed and concentrated again. Separately, a solution of silver nitrate was prepared. 70 mL of the concentrated silver nitrate solution was mixed with 30 mL of the concentrated mixture of the plant extracts. The beaker was then put in a mechanical stirrer. The beaker was then put in the magnetic shaker for 24 hours. The solution was then put into centrifuge tubes and centrifuged for 10 minutes. The supernatant was removed, and the nanoparticle concentrated solution was collected in one test tube. This is the end result of the nanopaint. Die stone samples were tested for two most commonly found bacteria in the oral cavity (Streptococcus mutans and Lactobacillus). These were incubated for 24 hours, and the results were observed. Results and Discussion: The zones of inhibition after 24 hours of incubation were observed and the die stone samples which were freshly dipped in the nanopaint, were found to have the maximum activity in comparison to the dipped and dried sample and the control sample. Independent t test was conducted and the P values are statistically significant (P value < 0.05). The mean values for freshly dipped nanopaint samples (19 ± 1.0 for Lactobacillus and 15.33 ± 1.155 for S. mutans) were higher than dried samples (11 ±1.0 for Lactobacillus and 10 ± 1.0 for S. mutans), for both bacteria tested against them. This proves that even though both nanopaint groups have good antimicrobial property, the freshly dipped nanopaint is more effective. Conclusion: Die stone immersed in nanopaint, where the nanoparticle is silver nanoparticle incorporated with green tea and neem, shows good antimicrobial properties which will help in reducing the spread of infection in patients through dentists.
Keywords: Silver nanoparticles, Green tea, Neem, Die stone, Nanopaint, Antimicrobial effect,innovation

1. Introduction

The aim of this study is to evaluate and analyse the antimicrobial activity of nanopaint immersed die stone, where the nanoparticle used is silver nanoparticle incorporated with green tea and neem. The nanopaint is tested on samples of die stone. Dental gypsum is widely used and studied for its use in obtaining dental casts [1–3]. Plaster and stone products used in dentistry are made by calcining calcium sulfate dihydrate. The principal constituent of gypsum-based products is calcium sulfate hemihydrate,[4,5] (Ca SO4)2·H2O. Popularity of type IV gypsum is attributed to its ease of use, relatively quick setting, and reasonable accuracy [4]. In dentistry, die is very relevant owing to its use in studying and working models [6]. Dental models are subjected to constant handling, and hence, they must be fracture resistant for safe laboratory procedures [7]. The surface properties of the die stone influence its ability to tolerate all types of

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forces during a restoration [8]. Many dental restorations and appliances are constructed outside the patient's mouth using models and dies which should be accurate replicas of the patient's hard and soft tissues [9]. To obtain more accurate and durable dental casts, some alternative systems have been suggested such as die metallization, synthetic die, epoxy resin, [10] [11] [12] polyurethane resin reinforced stone, and electroformed dies. [13] [14]

The oral cavity, or mouth, includes several distinct microbial habitats, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard palate, and soft palate. There are many microbes naturally present in the oral cavity of both dentulous and edentalous patients. Some of the common genuses of bacteria throughout the mouth are Streptococci, Neisseria, Fusobacterium, Prevotella, and other anaerobic bacteria. Anaerobic bacteria in the oral cavity include: Actinomyces, Arachnia, Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus, Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacterium, Selenomonas, Treponema, and Veillonella.

The clinical problem with using die stone arises when the cast along the FPD try in goes to the dental clinic, and returns to the dental laboratory after the try in. After the try in, the FPD is placed back onto the cast, due to which the bacteria from the oral cavity of the patient gets transferred to the cast. Since the cast is sent to the laboratory, there is a chance of cross infection spreading from the dental clinic to the laboratory. This leads to the transfer of infection to the laboratory technicians. Contamination of dental casts can occur via direct contact with impression materials that are contaminated by patients fluids. Thus, the development of dental stone with antimicrobial activity to reduce cross-contamination between patients and laboratory personnel is needed. The aim of this study is to evaluate and analyse the antimicrobial activity of nanopaint immersed in die stone, where the nanoparticle used is silver nanoparticle incorporated with green tea and neem.

2. Materials and Methods

The method by which this study was done, is divided into 3 main parts, after which statistical analysis was done. These are, nanopaint preparation, sample preparation and testing of nanopaint. The following steps were employed to check if die stone immersed in nanopaint has any antimicrobial effect. Statistical analysis was done using SPSS software, and the results were obtained by doing independent t tests.

Nanopaint Preparation

First, a concentrated solution of neem was prepared using neem extract powder. Similarly, in the same concentration, a green tea solution was also prepared in another beaker. Both of these were filtered, mixed and concentrated again. Separately, a solution of silver nitrate was prepared and concentrated. 70 mL of the concentrated silver nitrate solution was mixed with 30 mL of the mixture of the plant extracts. The beaker was then put in a mechanical stirrer. Readings were taken using a spectrophotometer at regular time intervals. The beaker was then put in the magnetic shaker for 24 hours and a final spectrophotometer reading was taken to analyse the nanoparticle size and amount. The solution was then put into centrifuge tubes and centrifuged for 10 minutes. The supernatant was removed, and the nanoparticle concentrated solution was collected in one test tube. This is the end result of the nanopaint.

Small and equally sized samples of die stone of diameter 6mm and thickness 2mm were prepared using a mould, so that the nanopaint could be tested on them.the company used for gypsum product was "Kalabhai Ultrarock Die Stone" and the water:powder ratio was 0.6 (60:100).

Testing of the Nanopaint

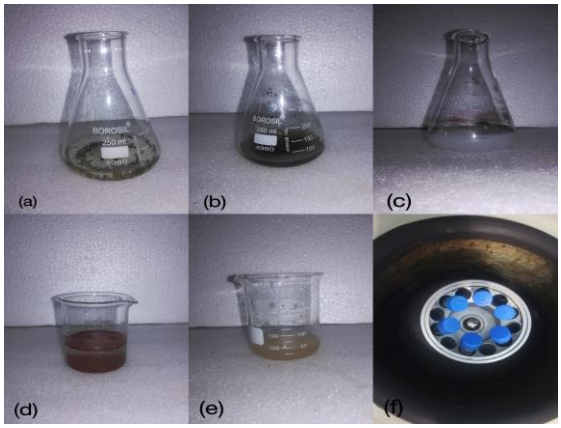

Figure 1. (a)Concentrated green tea extract ; (b)Concentrated neem extract ; (c)Concentrated Silver Nitrate extract ; (d)Mixture of concentrated green tea and neem extracts ; (e)Nanopaint solution prepared after using mechanical stirrer ; (f)Centrifugation of nanopaint, done to obtain concentrated nanopaint solution

Sample Preparation

The die stone samples were tested for two most commonly found bacteria in the oral cavity (Streptococcus mutans and Lactobacillus). In one Petri dish, three cavities were made. One for a control sample, which is not dipped in the nanopaint. One is for a freshly dipped sample and the third one is for a dipped and dried sample. These were incubated for 24 hours and the results were observed. The nanopaint was also coated on a die stone sample and was tested using a propellometer for its surface roughness and thickness.

3. Statistical Analysis

Statistical analysis was done using SPSS software version 20.0. Independent t test was done to compare the two groups of nanopaint (group 1 - dried nanopaint, group 2 - fresh nanopaint) based on their antimicrobial activity on different bacteria (Lactobacillus and Streptococcus mutans).

4. Result

The Petri dishes were studied after incubation of 24 hours and the zones of inhibition were measured. A caliper that measures in millimeters was taken and the "0" was placed in the center of the antibiotic disk. The area with zero growth was measured from the center of the disk to the edge of the area.

Following are the zones of inhibition

(b) zones of inhibition for the growth of Streptococcus mutans

Independent t test was conducted in two groups namely, group 1 - dried nanopaint, group 2 - fresh nanopaint. For lactobacillus, group 2 showed a better result, as its mean value (19 ± 1.0) is higher than group 1 (11 ± 1.0), which is also considered to be a good result. The difference between these two groups is considered to be statistically significant as the P value is 0.001 (P value < 0.05). For S. mutans, group 2 showed a better result, as its mean value (15.33 ± 1.155) is higher than group 1 (10 ± 1) , which is also considered to be a good result. The difference between these two groups is considered to be statistically significant as the P value is 0.004 (P value < 0.05).

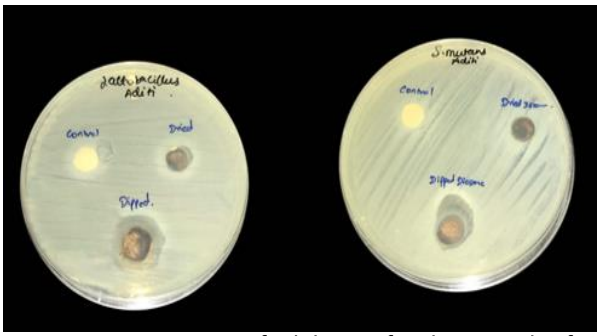

Figure 2. (a) zones of inhibition for the growth of Lactobacillus,

Table 1. Table showing comparison between two groups (group 1 - dried nanopaint, group 2 - fresh nanopaint) based on their antimicrobial activity on different bacteria (Lactobacillus and Streptococcus mutans)

Bacteria	Group	Mean ± Standard Deviation	Standard Error	95% Confidence Interval		t	df	P value
Lactobacillus	1 (NP Dried)	11 ± 1.0	0.577	10.26	5.73	9.798	4	0.001*
	2 (NP Dipped)	19 ± 1.0	0.577	10.26	5.73			
S. mutans	1 (NP Dried)	10 ± 1.0	0.577	7.78	2.88	6.047	4	0.004*
	2 (NP Dipped)	15.33 ± 1.155	0.667	7.8	2.86			
*P Value derived from independent t test, significant (P value < 0.05)								

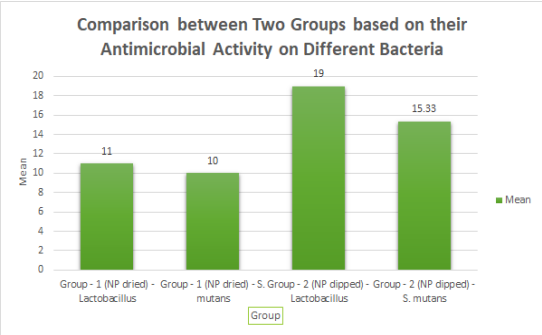

Figure 3. Bar graph showing comparison between two groups (group 1 - dried nanopaint, group 2 - fresh nanopaint) based on their antimicrobial activity on different bacteria (Lactobacillus and Streptococcus mutans)

5. Discussion

It can be observed that the zone of inhibition is the maximum for the die stone samples which were freshly dipped in the nanopaint solution and inserted in the Petri dish. The die stone sample which was dipped and dried has medium to good efficiency as an antimicrobial agent. The control group, because it was not dipped at all in the nanopaint solution, has 0 activity. It was also observed that the zones of Inhibition are more for Lactobacillus than for Streptococcus mutans. This study was chosen specifically to target the antimicrobial property shown by the three main components of the nanopaint solution i.e., silver nanoparticles, green tea and neem. Using their antibacterial property, the spread of infections from the patient to the dental laboratory technicians can be prevented. These three constituents I.e., silver nanoparticles, green tea, and neem, were chosen because they have their own individual antimicrobial properties. Silver nanoparticle brings change in functional proteins in plasma membrane as well as retarded DNA replication, attack respiratory chain resulting in death of microbe cell. Bacteria give a tough time as compared to fungi [15]. Silver nanoparticles work best against bacteria and fungi as today medicines worked such as streptomycin and penicillin against bacteria and fungi respectively [16]. The mechanism of action of the antibacterial activity of AgNPs is attacking the respiratory chain and cell division that ultimately leads to cell death[17]. The silver nanoparticles have also been reported to release silver ions inside the bacterial cells, further enhancing their bactericidal activity [18]. Green tea is known to contain catechins polyphenols which have a direct antimicrobial property by damaging cell membrane, inhibiting fatty acid synthesis and enzyme activity[19]. Many of the direct effects of tea catechins are a result of the catechins binding to the bacterial lipid bilayer cell membrane, which then causes damage to the membrane. This damage can then lead to a variety of related antimicrobial effects [20]. Bacterial cell membrane damage inhibits the ability of the bacteria to bind to host cells [21], and inhibits the ability of the bacteria to bind to each other to form biofilms, which are significant in pathogenesis [22]. Bacterial membrane damage also results in an inability of the bacteria to be able to secrete toxins[23,24]. Neem has a property of disrupting the integrity of the bacterial cell membrane [25].

In an experiment done by Irshad A. Wani, results

suggest very good antimicrobial activity of the silver nanoparticles against the test microbes [26]. This coincides with our research and proven fact of silver nanoparticles having good antimicrobial properties. Green tea has been shown to have an antibacterial property, as proved in an article, which reported that tests conducted showed that the antimicrobial effects of green tea have shown that the potential for preventive and therapeutic purposes is present [27]. The research paper "Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro", reports that the formulated neem nanoemulsion showed antibacterial activity against the bacterial pathogen *Vibrio vulnificus* by disrupting the integrity of the bacterial cell membrane [28]. This is consistent with our research which also proves the antimicrobial effect of green tea and neem. Another study done by Goda P., states that neem leaf extract has a significant antimicrobial effect against E. faecalis and C. albicans. It has demonstrated that microbial inhibition potential of neem leaf extract observed in this study opens perspectives for its use as an intracanal medication[29]. In a previously published article [30], Type IV dental stone incorporated with 4 types of disinfectants; Diamond Rock D (3-iodo-2-propynylbutylcarbamate), Diamond Rock B (zeolite), Diamond Rock Z (thiabendazole) and Diamond Rock T (2-benzimidazole carbamic acid), were tested against bacteria like Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. The incorporated dental stone samples proved to have antimicrobial property against these bacteria, and the incorporation also increased the compressive strength of the dental stone. Another study [31] showed that type III and type IV gypsum products, when mixed with disinfectants such as 0.525% sodium hypochlorite and 2% glutaraldehyde, in order to produce antimicrobial property, decreased the strength of the dental stone considerably. A research paper, [32] conducted tests on dental stone after incorporating 4 disinfectants in 4 samples namely, glutaraldehyde, povidone-iodine, chlorhexidine and sodium hypochlorite. Out of these, only glutaraldehyde and povidone-iodine killed all contaminating microorganisms within 1 hour. Two percent glutaraldehyde was the most effective disinfectant with the least adverse effects on the physical properties of the set cast. Although povidone-iodine caused a decrease in the compressive strength of the set cast, it can be considered to be a sound alternative.

This study is better than the other research papers published previously, because instead of incorporating the solution as a into the powder for mixing, which has proven to lower the strength of the gypsum powder and alter its properties, this nanopaint is sprayed on the cast. It can also be painted. This will inhibit the nanoparticles from interfering between the bonding of the dental stone powder and not lower the compressive and tensile strength of the cast. The nanopaint was tested using a profilometer, which showed that the nanopaint solution does not have any dimensional effect, which means that it does not change the dimension of the cast because it forms a thin uniform layer which is dimensionally insignificant. It will only show its antimicrobial property and will not have any effect on the treatment procedure, or the appliances/prosthesis prepared for the patient. This nanopaint solution can last upto 2 weeks and then has to be replaced with a newly made nanopaint. The limitation with the prepared nanopaint, is the short duration of viability. Since it is composed of natural plant extracts, there are chances of it decomposing after a duration of 2 weeks. After which, new or freshly made nanopaint will have to be prepared. However, further investigation on other properties such as dimensional stability, detail reproduction are still needed. Further studies and experiments are needed to test and increase the duration of viability of the nanopaint, for maximum usage.

6. Conclusion

Using this nanopaint solution where the nanoparticle is silver nanoparticle incorporated with green tea and neem, we can prevent the spreading of infections from patients via doctors to the lab technicians. While using any casts during treatments, the cast can be dipped in the solution, by the dentist, before sending it to the laboratory.

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