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To assess the efficacy of titanium dioxide nano spray on disinfection of endomotor: A microbiological study

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Abstract
The dental operatory poses possible modes of transmission of nosocomial infections. Infection control is a major issue in dental practice, where contamination with blood, saliva and aerosol is bound to occur. The most recent disease to be classified by the World Health Organization as a pandemic, COVID-19 joins cholera, smallpox and influenza as among the most brutal killers in history. According to Guidelines for Infection Control in Dental Health Care Settings 2003- the 'Environmental Infection Control for Clinical Contact Surfaces', it is recommended to clean and disinfect clinical contact surfaces that are not barrier protected using an EPA registered hospital disinfectant. Therefore, the objectives of this study were to determine the level of bacterial contamination of Endomotors among endodontists involved in patient care and to determine the usefulness of disinfecting with Titanium Dioxide Nano spray compared to isopropyl alcohol.

Keywords: Titanium dioxide, disinfection, dental operatory, endomotor

1. Introduction
The cross contamination between the dental team and the patient can be reduced with the practice of universal precaution-treating every patient seeking dental treatment as potentially infected. Use of proper barrier techniques-Personal Protective Equipments such as protective gloves, face masks, mouth masks, protective eye wear, protective gowns, and aprons [2]. However, to avoid transmission of infection between patients, the instruments are subjected to sterilization procedures such as autoclaving and disinfectants for the non autoclavable ones. Endomotors are such equipments that are non autoclavable and have proved to be a quintessential part of an endodontist's life. As much as we tend to use it on a daily basis in endodontic treatment, we forget how important it is to disinfect the control unit. Contra angle endodontic hand piece is autoclavable, but the control unit body and motor attachment is not.
Manufacturers have recommended the use of cotton cloth moistened with soap water or alcohol wipes in the form of ethyl or isopropyl alcohol to disinfect the exposed surfaces of endomotors [5]. However, these disinfectants evaporate quickly as they are volatile and their effect decrease substantially due to lack of substantivity. Dayane de Melo Costa *et al*. in their study have shown that the use of alcohol for wiping contaminated instruments caused fixation of micro-organisms increasing difficulty in cleaning due to drying, with little effect on bacterial viability [6]. The discovery of photocatalytic property of TiO2 by Dr. Akira Fujishima in 1967 initiated a revolution. Titanium dioxide Nano spray has been used in ICUs and hospital furnishing since long with good disinfection results. Palaniswamy U *et al*. have shown that mobile phone decontamination with the use of TiO2 showed antimicrobial efficacy lasting upto 1week. However, there haven't been studies testing the disinfection efficacy of TiO2 NS on endomotors and their comparison with standard isopropyl alcohol disinfection. The null hypothesis is that there will be no difference in the disinfection efficacy between isopropyl alcohol and Titanium dioxide nano spray.

2. Materials and Methods
2.1 Inclusion Criteria: Endomotors minimum usage of 3 months in a dental clinical set up.

2.2 Exclusion Criteria: Unused or new endomotors

2.3 Objectives

- Determine bacterial contamination of Endomotors amongst endodontists' involved in patient care
- Compare the disinfection of Endomotors using Titanium Dioxide Nano spray and Isopropyl alcohol.

2.4 Materials

40 Endomotors, TiO2 Nano spray (Armor 8, Armor Life Sdn Bhd, Kuala Lumpur, Malaysia), Isopropyl alcohol, Tris-glycine buffer, Sterile cotton tip applicators, Sterile test tubes, Sterile gauze, Sterile saline.

2.5 Methodology

40 Endomotors were divided into following 4 groups based on the mode of disinfection:

1. Group 1- Isopropyl alcohol group
   a. Group 1a- control group- swabs without disinfection
   b. Group Ib- swabs after disinfecting with isopropyl alcohol
2. Group 2- TiO2 NS group
   a. Group 2a- control group- swabs without disinfection
   b. Group 2b- swabs after disinfecting with TiO2 spray

Cotton tip applicators moistened with sterile saline was rotated on the surfaces of Endomotors for control group 1a and 2a and stored in test tubes containing tris-glycine buffer.

For group 1b, the surfaces were then wiped with 70% isopropyl alcohol waited for 10 min. thereafter, swabs were taken.

For group 2b, the surfaces were then sprayed with TiO2 NS, allowed to dry for 10 minutes, then wiped with sterile gauze and swabs were taken.

All the swabs were collected in test tubes containing tris glycine buffer as transport media.

100 ul of each of the samples from test tubes were poured into the sterilized labelled petri plate and spread thoroughly.

25ml of Heme agar was poured on the samples and allowed to solidify using pour plate method.

After solidification, the plates were incubated at 37 °C for 24 hours.

2.6 Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Wilcoxon Signed Ranked Test was used to compare the mean CFUs between different time periods with respect to Isopropyl and Titanium dioxide NS. The level of significance [P-Value] was set at $P< 0.05$.

3. Results and Discussion

In the present study, the endomotors were collected after at least 3 months of usage and it was assumed that the dentists were following the disinfection protocols for endomotors according to the manufacturer's instructions. The Mean bacterial CFU before isopropyl alcohol wipe was 115.65x104 and before TiO2 spray was 112.95x104 with no statistical significant difference. This shows that even after routine disinfection and cleaning of endomotors, there was significant amount of bacterial load seen.

However, the mean bacterial CFU after isopropyl alcohol wipe was 53.05x104 and after TiO2 spray was found to be 16.05x104, which was statistically significantly different. It was observed in our study that, even after cleaning with 70 percent isopropyl alcohol swabs, endomotors carried larger CFU bacteria compared to the bacterial count after disinfecting with TiO2 NS. This can be attributed to the unique photocatalytic and self-sterilising properties of Titanium dioxide. Titanium dioxide nano spray which is used in the present study, has been tested for its antibacterial efficacy in previous studies. According to a study conducted by Udayakumar Palaniswamy, there was a significant reduction in the mean number of CFUs after decontamination with TiO2 nano spray which was effective even after 1 week. The mean CFU counts at 10 min and 1 week after TiO2 NS were 236.00 ± 187.50, 17.98 ± 24.55, and 53.10 ± 79.00 respectively, thereby reducing the bacterial load by 78% even after 1 week of TiO2 NS use.9 Discovery of Titanium dioxide's photocatalytic and super hydrophilicity provides cleaning and self-sterilising property. It has been incorporated into tiles that cover the floor and walls of operating rooms, in silicone rubber of medical catheters and hospital garments and uniforms.

The photocatalytic property of TiO2 was discovered by Dr. Akira Fujishima in 1967. When titanium dioxide (TiO2) was irradiated with light, the energy absorbed enabled the decomposition of water into hydrogen and oxygen. This Photocatalytic water decomposition was later called the Honda-Fujishima effect which lead to the inactivation of pathogenic micro-organisms.7 Because TiO2 is a semiconductor, it can excite pairs of electrons. The photogenerated electrons then react with molecular oxygen (O2-) to produce superoxide radical anions (O2−) and the photogenerated holes react with water to produce hydroxyl (-OH) radicals. These two reactive radicals then work together to decompose organic compounds. The longer the film is illuminated with UV light better will be decomposition of organic matter. TiO2 NS in the present study was sprayed and allowed to dry for 10min. This helped in decomposition of the organic compounds of the pathogenic bacteria present. Also, unlike the volatile nature of isopropyl alcohol, TiO2 NS showed substantivity and better antibacterial action than isopropyl alcohol.

3. Tables and Figures

Table 1: Comparison of mean CFUs (x 10^4) before and after Isopropyl alcohol wipe using Wilcoxon Signed Ranked Test

Time	N	Mean	SD	Mean Diff	P-Value
Before	20	115.65	10.71	62.60	<0.001*
After	20	53.05	8.99		

Table 2: Comparison of mean CFUs (x 10^4) before and after Titanium Dioxide spray using Wilcoxon Signed Ranked Test

Time	N	Mean	SD	Mean Diff	P-Value
Before	20	112.95	12.56	96.90	<0.001*
After	20	16.05	5.65		

Table 3: Comparison of mean CFUs (x 10^4) before spraying Isopropyl alcohol and Titanium Oxide using Wilcoxon Signed Ranked Test

Spray	N	Mean	SD	Mean Diff	P-Value
Isopropyl	20	115.65	10.71	2.70	0.12
Ti. Oxide	20	112.95	12.56		

Table 4: Comparison of mean CFUs (x 10^4) after Spraying Isopropyl and Titanium Oxide using Wilcoxon Signed Ranked Test

Spray	N	Mean	SD	Mean Diff	P-Value
Isopropyl	20	53.05	8.99	37.00	<0.001*
Ti. Oxide	20	16.05	5.65		

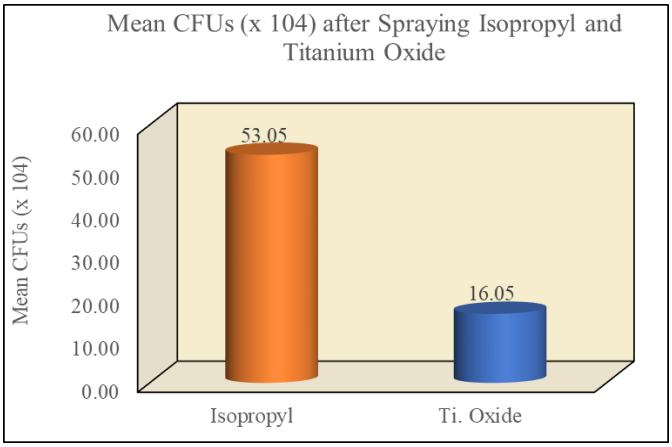

Fig 1: Mean cfu after disinfecting with isopropyl alcohol and after titanium dioxide nano spary

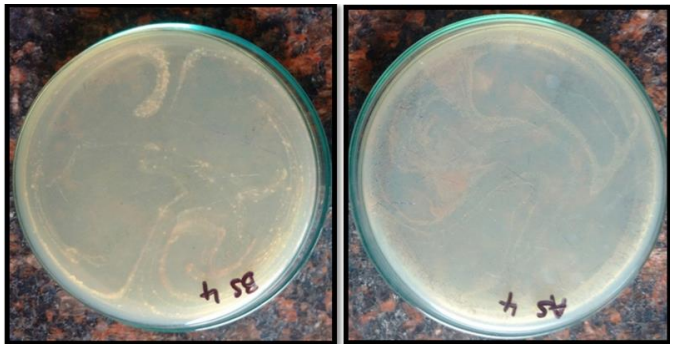

Fig 2, 3: bacterial colonies before and after disinfection with isopropyl alcohol

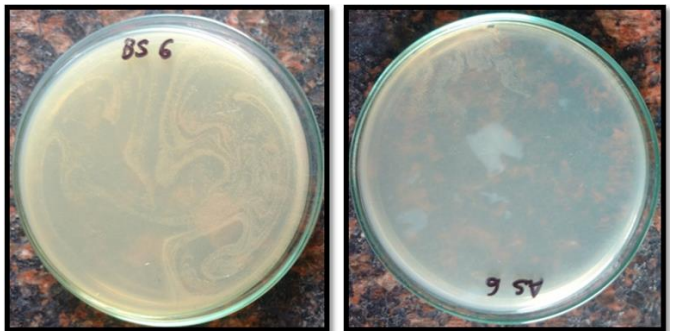

Fig 4, 5: Bacterial colonies before and after disinfection with titanium dioxide nano spray

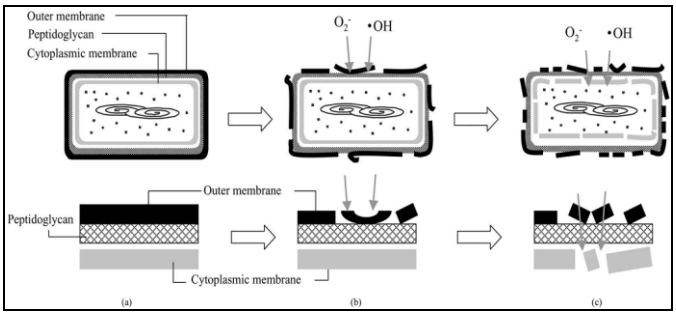

Fig 6: Process of E. coli photokilling on TiO2 film

4. Conclusions (Times New Roman, 12, Bold)

Under the limitations of this study it can be concluded that: Titanium Dioxide nano spray can serve as a better disinfectant than isopropyl alcohol to disinfect endomotor control unit. Titanium Dioxide can also be used to effectively disinfect exposed surfaces of other dental equipments, dental chair and upholstery.

5. Acknowledgments

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