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Original Article

Dentistry Section

Comparative Evaluation of Antibacterial Efficacy of Two Bioceramic Root Canal Sealers Incorporated with Novel Silica Doped TiO_2 Nanoparticles: An In-vitro Study

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ABSTRACT

Introduction: The success of Root Canal Treatment (RCT), depends on the complete elimination of microorganisms in combination with complete root canal system closure. Complete elimination of bacteria cannot be done by cleaning and shaping alone from lateral canals, isthmuses, and apical deltas. Thus, root canal sealers with ideal physical, biological, and improved antimicrobial characteristics are thus, necessary to avoid reinfections.

Aim: To evaluate the antibacterial efficacy of two Bioceramic (Bio-C) modified root canal sealers, using Silica Doped Titanium Dioxide Nanoparticles ($SiTiO_2$), a unique, extremely effective antibacterial agent on root canal dentin infected with Enterococcus faecalis (*E. faecalis*).

Materials and Methods: This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Department of Microbiology at Sree Sai Dental College and Research Institute, Andhra Pradesh, India. The study was done in August 2022. The antibacterial efficacy of the two Bio-C sealers was evaluated by counting Colony Forming Units (CFU) and the percentage of live bacteria by a confocal laser scanning microscope. A total of 60 middle thirds of single-rooted teeth incubated with *E.faecalis* were chosen. Gutta-percha (GP) was used to fill the canals along with sealers in all six groups (group I-VI), namely Mineral Trioxide Aggregate based (MTA Fillapex); MTA Fillapex+$SiTiO_2$ NPs (1% wt); MTA Fillapex +2% wt $SiTiO_2$ NPs; Bio-C; Bio-C+ 1% wt $SiTiO_2$ NPs; Bio-C+ 2% wt $SiTiO_2$ NPs respectively, and incubated for seven days, each tooth was divided into two halves longitudinally. Microbiological analysis was conducted on one half, and microscopic analysis on the other half. The six groups were compared using a one-way Analysis of Variance (ANOVA) and intergroup comparison with Tukey's post-hoc tests.

Results: The addition of SiTiO2 NPs to Bio-C and MTA Fillapex significantly reduced the bacteria, compared to an unmodified sealer (p-value<0.001). MTA Fillapex with $SiTiO_2$ NPs showed higher bacterial viability compared with Bio-C with $SiTiO_2$ NPs.

Conclusion: Loading endodontic sealers with $SiTiO_2$ NPs has a material-dependent impact on the antibacterial properties, that could lower the frequency of secondary infections.

Keywords: *Enterococcus faecalis*, Mineral trioxide aggregate, Titanium dioxide

INTRODUCTION

The goal of endodontic therapy is to completely eradicate microorganisms, with the aid of mechanical cleaning and shaping, antibacterial irrigants, and sufficient filling of the empty space. However, these procedures do not result in total root canal disinfection. *E.faecalis* is commonly isolated from teeth with long lasting endodontic infections. The ability of *E.faecalis* to survive for long periods without nutrition and the presence of several virulence factors contribute to their persistence. Therefore, to enhance their antibacterial activity and prevent reinfection, antimicrobial agents are added to root canal sealers [1]. The evolution of novel, efficient antibacterial agents appears to be of utmost important. Silica is a potent and biocompatible antimicrobial agent with no known antibacterial resistance documented. Due of the increasing surface area, SiO_2 activity would become more relevant at the nanoscale. Si nanoparticles, according to research by Ghanbari H et al., prevented bacteria from adhering to oral biofilms [2].

Utilising nanoparticulate materials to disinfect root canals has been a subject of recent research [3]. Titanium dioxide Nanoparticles (TiO_2NPs) shows an antibacterial effect due to their physicochemical and biological activities in addition to their unique photocatalytic action [4]. Previous studies have investigated the antibacterial effect of silica and titanium dioxide nanoparticles incorporated into many biomaterials like composites, irrigants, bleaching agents, and implant surfaces and this has shown, sustained release rates with the ability to reduce bacterial colonisation, biocompatibility, as well as, chemical bonding to dentin [5,6]. There are a few investigations about the antibacterial properties of bioceramic sealers like MTA Fillapex and Bio-C sealer. These have been emphasised because of their alkaline pH, sealing ability, biocompatibility, dimensional stability, the potential to increase root strength by osteogenic constituents like calcium phosphate and calcium silicate [7,8]. However, there is no study in the available literature, assessing the comparative antimicrobial effectiveness of these sealers incorporated with $SiTiO_2$ NPs.

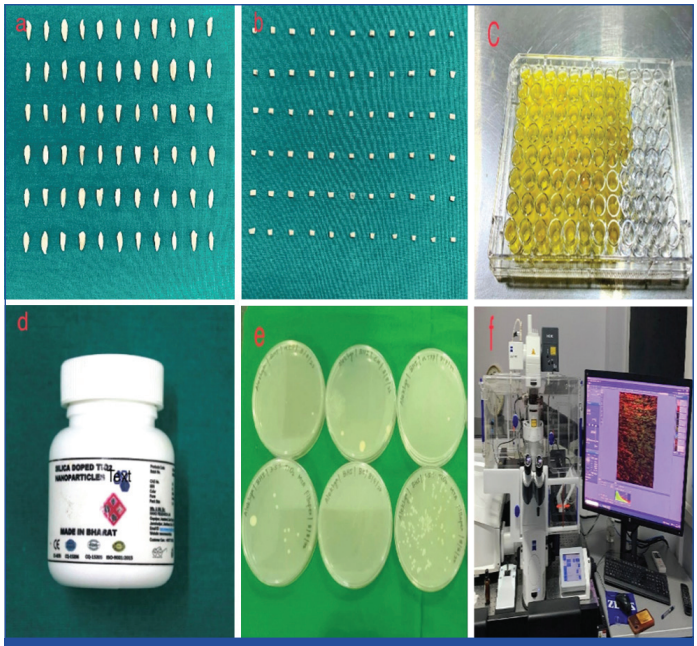
Thus, the present study was performed to compare and evaluate the antibacterial efficiency of Bio-C root canal sealers, that had been modified with a highly loaded, antimicrobial agent $SiTiO_2$ NPs on root canal dentin, that had been infected with *E.faecalis*.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Department of Microbiology at Sree Sai Dental College and Research Institute, Andhra Pradesh, India. The study was done in August 2022. Ethical approval was obtained from Institutional Ethics Committee (SSDCRI/IEC/2022/5/S1).

Inclusion criteria: Single-rooted teeth with a mature apex were included in the study.

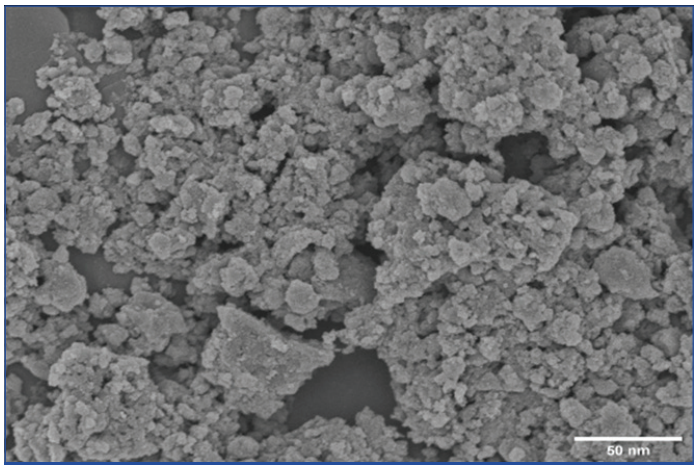
Exclusion criteria: Teeth having fractures, root caries, morphological deformities, or severely curved roots were excluded from the study [Table/Fig-1].



[Table/Fig-1]: a) A 66 single-rooted teeth; b) Middle third of each root; c) Canals were infected with *E.faecalis* American Type Culture Collection (ATCC) in microtiter plates; d) $SiTiO_2$ NPs; e) Microbiological analysis; f) Microscopic analysis (confocal laser scanning microscope).

Study Procedure

The antibacterial efficacy of two Bio-C root canal sealers incorporated with $SiTiO_2$ NPs were evaluated using microbiological and microscopic analysis [Table/Fig-2], $SiTiO_2$ NPs (Nano Research Laboratory; Jamshedpur, India) were gradually added to Bio-C sealer at either 1% or 2% wt (Angelus, Paraná, Brazil), or MTA Fillapex (Angelus, Londrina, Brazil). These $SiTiO_2$ NP concentrations were determined following a pilot research, that evaluated the flow of materials with concentrations from 5% to 1% wt; the addition of $SiTiO_2$ NPs in the 1%-2% range had no impact on the setting of the materials [9].

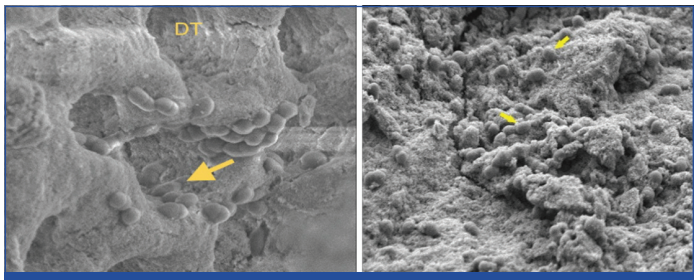


[Table/Fig-2]: Scanning Electron Microscope (SEM) images of $SiTiO_2$ NPs with scale bar 50 nm.

Sample preparation: A total of 60 single-rooted teeth were collected and the middle third of each root was obtained by sectioning them using a diamond disc. ProTaper rotary files upto size F3 were used to prepare the root canals. Finally, 5% sodium hypochlorite and 17% Etheylenediamine Tetraacetic Acid (EDTA) were used to irrigate canals and autoclaved for 20 minutes at 121°C.

Root canal infection with Enterococcus faecalis: Canals were infected with *E.faecalis* (ATCC 29212) in the present study. In a 96-well microtiter plate, prepared root samples were placed. 20 µL of *E.faecalis* culture with 300 µL of tryptone soya broth was injected into each well, then incubated for three weeks anaerobically at 37°C. For three weeks, the growing media was replaced every other day. All the procedures were done under a laminar flow hood.

Confirmation of tooth contamination: The samples were rinsed with 5 mL of sterile saline, following the incubation period. Six root samples (one from each group) were viewed under a SEM to verify the presence of bacteria and their structure [Table/Fig-3].



[Table/Fig-3]: Scanning Electron Microscope (SEM) images of dentin surface at high magnification, dentinal tubules are occluded by *E.faecalis*.

Group specification: The remaining 60 samples were divided into six groups (10 in each group) and canals were filled with GP and sealers:

Group I- MTA Fillapex

Group II- MTA Fillapex + $SiTiO_2$ NPs (1% wt)

Group III- MTA Fillapex + $SiTiO_2$ NPs (2% wt)

Group IV- Bio-C

Group V- Bio-C + $SiTiO_2$ NPs (1% wt)

Group VI- Bio-C + $SiTiO_2$ NPs (2% wt)

Samples were incubated anaerobically for seven days at 37°C. After the incubation period, each tooth was split into two halves; both, microscopic and microbiological analyses were conducted on separate halves of the sample.

Microbiological analysis: Gates-Glidden drills were used to obtain dentin powder from the canal. The samples were all transferred to 2 mL of Brain Heart Infusion (BHI) media and incubated for another 24 hours at 37°C. In microcentrifuge, test tubes containing 1 mL BHI, the resulting dentin powder was collected, vortexed, serially diluted, and plated. At 37°C, the plates were incubated for 48 hours. The Colony Forming Unit (CFU) were calculated and converted to their log10 values [10].

Microscopic analysis: Near the pulp canal space, a 1 mm thick portion was obtained from each dentin block using IsoMet 4000 Precision Saw. The root sections were stained with 0.01% acridine orange (green fluorescence) and 0.01% propidium iodide (red fluorescence) and then rinsed with phosphate-buffered saline. Specimens were transferred to glass coverslips and Zeiss Laser Scanning Microscopy (LSM) 880 Airyscan (Carl Zeiss, Oberkochen, Germany) data were analysed immediately to ascertain the proportion of live/dead cells for each treatment. Image J software was utilised to calculate the proportion of live (green fluorescence) and dead (red fluorescence) cells [11].

Flow test: According to standardised International Organisation for Standardisation (ISO) 6876:2012 protocols, the flow of stock and modified sealers was evaluated [12]. Applying 0.05±0.005 mL of sealer with a graduated syringe at the center of a glass plate. A second glass plate with an additional mass, a total of 120±2 g, was positioned in the center of the sealer at 180±5 seconds, following the start of the mixing process. After 10 minutes of the initial mixing time, the weight was taken off and the compressed sealer discs' maximum and minimum diameters were measured in millimeter (mm) using digital calipers. Final flow rate was calculated by subtracting the minor diameter from the major diameter [12].

STATISTICAL ANALYSIS

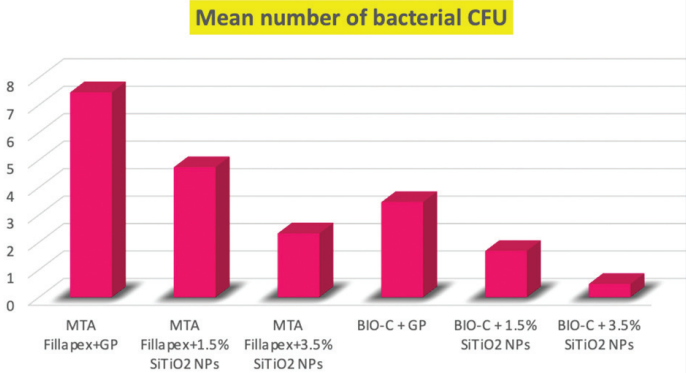
The statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 20.0. One-way ANOVA was used for the analysis, and Tukey's post-hoc test was used to compare groups. The level of was set at (p-value<0.05).

RESULTS

Microbiological analysis: Bio-C+2% $SiTiO_2$ NPs significantly reduced the CFU count, showing a mean of 0.48±0.672 log CFU/mL compared with Bio-C+ GP (3.46±0.614) log CFU/mL and Bio-C +1% $SiTiO_2$ NPs (1.68±0.277) log CFU/mL [Table/Fig-4,5]; MTA Fillapex+2% $SiTiO_2$ NPs showed an improved antimicrobial performance, showing CFU (2.32±0.804) log CFU/mL versus MTA Fillapex + 1% $SiTiO_2$ NPs (4.73±0.660) log CFU/mL and MTA Fillapex + GP (7.46±1.174). log CFU/mL. Bio-C sealer showed a better reduction of CFUs compared with the MTA Fillapex groups [Table/Fig-6].

Groups	n	Mean CFU/mL	SD	F-value	p-value
MTA Fillapex	10	7.46×10^8	1.174	55.060	<0.001
MTA Fillapex + $SiTiO_2$ NPs (1% wt)	10	4.73×10^8	0.660		
MTA Fillapex + $SiTiO_2$ NPs (2% wt)	10	2.32×10^8	0.804		
Bio-C	10	3.46×10^8	0.614		
Bio-C + $SiTiO_2$ NPs (1% wt)	10	1.68×10^8	0.277		
Bio-C + $SiTiO_2$ NPs (2% wt)	10	0.48×10^8	0.672		
Total	60	3.35×10^8	2.407		

[Table/Fig-4]: One-way ANOVA test applied for descriptive statistics of colony forming units in all six groups.
p-value <0.05 is statistically significant; SD: Standard deviation



[Table/Fig-5]: Schematic representation showing the number of viable bacterial cells (log CFU/mL) on root dentin after seven days of contact with MTA FA + GP, MTA FA + 1.5% $SiTiO_2$ NPs, MTA FA+3.5% $SiTiO_2$ NPs, Bio-C+ GP, Bio-C +1.5% $SiTiO_2$ NPs and Bio-C + 3.5% $SiTiO_2$ NPs group.

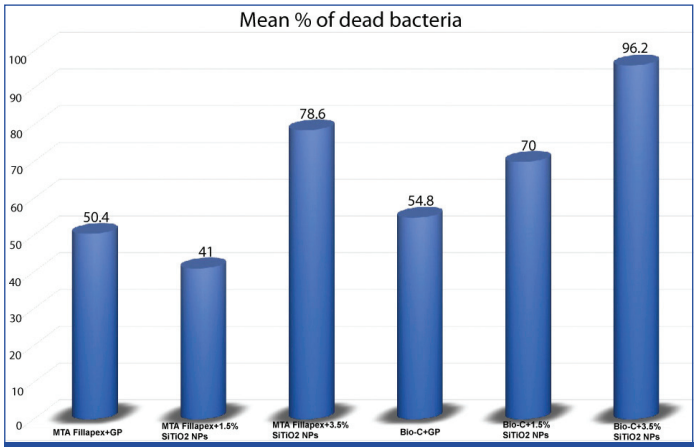
(I) Group	(J) Group	Mean difference (I-J)	p-value	95% Confidence interval	
				Lower bound	Upper bound
MTA Fillapex	MTA Fillapex + $SiTiO_2$ NPs (1% wt)	2.724*	<0.001	1.258	4.189
	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	5.140*	<0.001	3.674	6.605
	Bio-C	4.000*	<0.001	2.534	5.465
	Bio-C + $SiTiO_2$ NPs (1% wt)	5.780*	<0.001	4.314	7.245
	Bio-C + $SiTiO_2$ NPs (2% wt)	6.980*	0.001	5.514	8.445
MTA Fillapex +$SiTiO_2$ NPs (1% wt)	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	2.416*	0.001	0.950	3.881
	Bio-C	1.2760	0.114	-0.189	2.741
	Bio-C + $SiTiO_2$ NPs (1% wt)	3.056*	0.001	1.590	4.521
	Bio-C + $SiTiO_2$ NPs (2% wt)	4.256*	0.001	2.790	5.721
MTA Fillapex + $SiTiO_2$ NPs (2% wt)	Bio-C	-1.140	0.194	-2.605	0.325
	Bio-C + $SiTiO_2$ NPs (1% wt)	0.640	0.755	-0.825	2.105
	Bio-C + $SiTiO_2$ NPs (2% wt)	1.840*	0.008	0.374	3.305
Bio-C	Bio-C + $SiTiO_2$ NPs (1% wt)	1.780*	0.011	0.314	3.245
	Bio-C + $SiTiO_2$ NPs (2% wt)	2.900*	0.001	1.514	4.445
Bio-C + $SiTiO_2$ NPs (1% wt)	Bio-C + $SiTiO_2$ NPs (2% wt)	1.200	0.154	-0.265	2.665

[Table/Fig-6]: Tukey's post-hoc test was applied for pair-wise comparisons of colony forming units in all six groups; p-value <0.05 is statistically significant.

Microscopic analysis: Confocal images showed an increased percentage of dead bacteria in all the groups. The addition of $SiTiO_2$ NPs to MTA Fillapex and Bio-C sealers showed a significant reduction of live bacteria compared with the unmodified groups [Table/Fig-7,8]. The addition of 2% $SiTiO_2$ NPs to Bio-C sealer decreased the bacterial viability compared with 2% $SiTiO_2$ NPs to MTA FA at seven days incubation (4.8%±1.6% vs 46.2%±26.8%, p<0.05) [Table/Fig-9,10].

Groups	n	SD	Mean % of dead bacteria	F-value	p-value
MTA fillapex	10	5.899	50.40	4.231	0.007**
MTA fillapex + $SiTiO_2$ NPs (1% wt)	10	31.520	41.00		
MTA fillapex + $SiTiO_2$ NPs (2% wt)	10	4.506	78.60		
Bio-C	10	26.892	54.80		
Bio-C + $SiTiO_2$ NPs (1% wt)	10	34.205	70.00		
Bio-C + $SiTiO_2$ NPs (2% wt)	10	1.643	96.20		
Total	60	27.643	65.17		

[Table/Fig-7]: One-way ANOVA test applied for comparisions of mean percentages of dead bacteria in different sealers, **p-value <0.05 was considered as statistically significant.



[Table/Fig-8]: Schematic representation shows the mean percentage of dead bacteria, and group VI is having the highest antibacterial activity, followed by the group III, group V, group IV, group I and least for the group II.

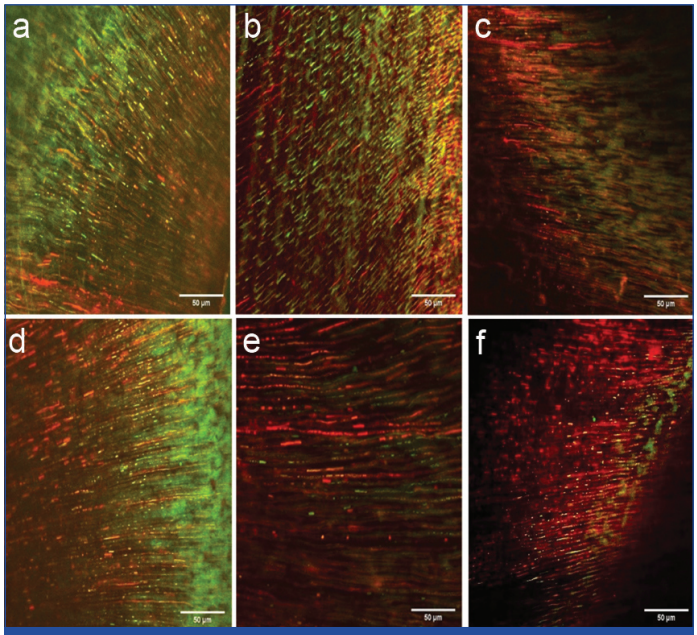
Flow test: With flow diameters of 17 mm, MTA Fillapex and Bio-C unmodified sealers, both were within the ISO 6876:2012 flow criteria [13]. $SiTiO_2$ NPs considerably reduced the flow of all modified sealers (p-value<0.05). For the MTA Fillapex, but not the Bio-C groups, the decrease in flow was associated with the mass of $SiTiO_2$ NPs present in the materials (p-value<0.05). All changed sealers were within the permissible ISO 6876 flow limits, with the exception of MTA Fillapex 2% wt $SiTiO_2$ NPs (15.7+0.35 mm) [Table/Fig-11,12].

DISCUSSION

Improved disinfection of the root canal system may be facilitated by enhancing and extending the antibacterial properties of sealers.

(I) Group	(J) Group	Mean difference (I-J)	p-value	95% Confidence interval	
				Lower bound	Upper bound
MTA Fillapex	MTA Fillapex + $SiTiO_2$ NPs (1% wt)	9.400	0.984	-33.92	52.72
	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	-28.200	0.365	-71.52	15.12
	Bio-C	-4.400	1.000	-47.72	38.92
	Bio-C + $SiTiO_2$ NPs (1% wt)	-19.600	0.727	-62.92	23.72
	Bio-C + $SiTiO_2$ NPs (2% wt)	-45.800*	0.034	-89.12	-2.48
MTA Fillapex + $SiTiO_2$ NPs (1% wt)	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	-37.600	0.116	-80.92	5.72
	Bio-C	-13.800	0.918	-57.12	29.52
	Bio-C + $SiTiO_2$ NPs (1% wt)	-29.000	0.335	-72.32	14.32
	Bio-C + $SiTiO_2$ NPs (2% wt)	-55.200*	0.007	-98.52	-11.88
MTA Fillapex + $SiTiO_2$ NPs (2% wt)	Bio-C	23.800	0.546	-19.52	67.12
	Bio-C + $SiTiO_2$ NPs (1% wt)	8.600	0.989	-34.72	51.92
	Bio-C + $SiTiO_2$ NPs (2% wt)	-17.600	0.805	-60.92	25.72
Bio-C	Bio-C + $SiTiO_2$ NPs (1% wt)	-15.200	0.883	-58.52	28.12
	Bio-C + $SiTiO_2$ NPs (2% wt)	-41.400	0.067	-84.72	1.92
Bio-C + $SiTiO_2$ NPs (1% wt)	Bio-C + $SiTiO_2$ NPs (2% wt)	-26.200	0.443	-69.52	17.12

[Table/Fig-9]: Pair-wise comparison of mean percentages of dead bacteria in different sealers by Tukey's post-hoc test; p-value <0.05 was considered as statistically significant.



[Table/Fig-10]: Representative confocal laser scanning microscopic images of the root canal dentin treated with: a) MTA FA; b) MTA Fillapex + 1% $SiTiO_2$; c) MTA FA + 2% $SiTiO_2$; d) Bio-C; e) Bio-C + 1% $SiTiO_2$; f) MTA Fillapex + 2% $SiTiO_2$ showing the remaining live (green) and dead (red) E.faecalis cells on dentinal tubules after seven days of medication.

However, the effects of incorporating $SiTiO_2$ NPs into a root canal sealer against *E.faecalis* impregnated into root dentin have never been reported. According to the results, both modified and unmodified sealers significantly reduced the number of bacterial cells in a dose-dependent manner. The highest antibacterial effect was reported in Bio-C + 2% $SiTiO_2$ NPs group followed by MTA Fillapex+2% $SiTiO_2$ NPs, Bio-C +1% $SiTiO_2$ NPs, Bio-C+GP, MTA Fillapex+1% $SiTiO_2$ NPs, and MTA FA+GP. Pair-wise comparisons between the groups showed significant differences (p-value<0.001).

Groups	Mean	SD	F-value	p-value
MTA Fillapex	22.6667	0.57735	200.05	<0.001
MTA Fillapex + $SiTiO_2$ NPs (1% wt)	18.6667	0.57735		
MTA Fillapex + $SiTiO_2$ NPs (2% wt)	15.0000	0		
Bio-C	25.6667	0.57735		
Bio-C + $SiTiO_2$ NPs (1% wt)	23.6667	0.57735		
Bio-C + $SiTiO_2$ NPs (2% wt)	20.0000	0		

[Table/Fig-11]: One-way ANOVA test applied for mean flow (mm) between six tested sealers.
p-value <0.05 is statistically significant

(I) group	(J) group	Mean difference (I-J)	p-value	95% Confidence interval	
				Lower bound	Upper bound
MTA Fillapex	MTA Fillapex + $SiTiO_2$ NPs (1% wt)	4.00000*	0.001	2.7072	5.2928
	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	7.66667*	0.001	6.3738	8.9595
	Bio-C	-3.00000*	0.001	-4.2928	-1.7072
	Bio-C + $SiTiO_2$ NPs (1% wt)	-1.00000	0.171	-2.2928	0.2928
	Bio-C + $SiTiO_2$ NPs (2% wt)	2.66667*	0.001	1.3738	3.9595
MTA Fillapex + $SiTiO_2$ NPs (1% wt)	MTA Fillapex + $SiTiO_2$ NPs (2% wt)	3.66667*	0.001	2.3738	4.9595
	Bio-C	-7.00000*	0.001	-8.2928	-5.7072
	Bio-C + $SiTiO_2$ NPs (1% wt)	-5.00000*	0.001	-6.2928	-3.7072
	Bio-C + $SiTiO_2$ NPs (2% wt)	-1.33333*	0.042	-2.6262	-0.0405
MTA Fillapex + $SiTiO_2$ NPs (2% wt)	Bio-C	-10.66667*	0.001	-11.9595	-9.3738
	Bio-C + $SiTiO_2$ NPs (1% wt)	-8.66667*	0.001	-9.9595	-7.3738
	Bio-C + $SiTiO_2$ NPs (2% wt)	-5.00000*	0.001	-6.2928	-3.7072
Bio-C	Bio-C + $SiTiO_2$ NPs (1% wt)	2.00000*	0.002	0.7072	3.2928
	Bio-C + $SiTiO_2$ NPs (2% wt)	5.66667*	0.001	4.3738	6.9595
Bio-C + $SiTiO_2$ NPs (1% wt)	Bio-C + $SiTiO_2$ NPs (2% wt)	3.66667*	0.001	2.3738	4.9595

[Table/Fig-12]: Pair-wise comparisons of flow by Tukey's post-hoc test in all six groups. p-value<0.05 is statistically significant.

Flow is a crucial component for root canal filling which enables the sealer to enter the imperfections of root canal networks. According to ISO 6876 criteria, all of the sealers provided flow rates that supported earlier research [14,15]. The Bio-C sealer demonstrated the maximum flow rate. The results of the culture-based microbiological research revealed that, when compared to the MTA Fillapex groups, Bio-C with or without $SiTiO_2$ NPs considerably decreased the quantity of bacteria. Literature shows that, in addition to its hydrophilic nature, and its active calcium hydroxide diffusion, for upto 21 days [7], Bio-C sealer can be alkaline, reaching a pH of 10. It is well known that a pH greater than nine can render microbial cell membrane enzymes inactive, which results in a reduction in biological activity or loss of the plasma membrane's integrity as reported by Allaker RP [16].

The antibacterial activity of the sealers was examined in the present study, while they were in contact with the root dentin. Due to its buffering properties and the binding of certain cationic compounds to dentin, dentin has been demonstrated to impede the antibacterial action of root canal medications, reducing their antimicrobial efficiency

[17,18]. *E.faecalis* was selected for the present study, because it is the most resistance and commonly appeared in the root canals of teeth with post-treatment apical periodontitis [19-22]. Numerous virulence factors have been implicated in the survival of *E.faecalis* following endodontic treatment. It is also capable of penetrating into dentinal tubules, as soon as, 48 hours after inoculation [23]. The gold standard for identifying and quantifying viable bacteria is CFU counting, using culture-based techniques. However, the accuracy of these techniques depends on the transport and culture medium, culture-based approaches are unable to identify live bacteria. The present study uses Confocal Laser Scanning Microscopy (CLSM) along with the fluorescent stains has made it possible to evaluate the structure, distribution, and viability of certain bacteria inside the biofilm [24,25].

Studies done by Shakya VK et al., (2016) and Mangat P et al., (2020) showed counts for $SiTiO_2$ NPs modified MTA Fillapex were less than the unmodified sealer and higher than Bio-C. It may be presumed that, despite its alkalinity and calcium release, the environment was still conducive to the life of the microorganism. Its proton pump is most likely a major contributing cause to its resistance to alkaline substances [26,27]. When compared to the effects of the unmodified sealers, the addition of $SiTiO_2$ NPs to both sealers (Bio-C and MTA Fillapex) considerably improved the antibacterial effect (p-value<0.001). The deeper penetration of nanoparticulate medications into the microbial cells and dentinal tubules is the primary goal of adding them to endodontic sealers. According to Waltimo T et al., adding SiNPs to dental biomaterials and cement has a favourable antibacterial effect through the release of ionic alkaline species over time and is being considered as a dentine disinfectant to provide an alternative to calcium hydroxide [28]. In recent years, researchers have explored the antibacterial properties of TiO_2 nanoparticles as an efficient antimicrobial agent against a large variety of species, including bacteria both gram-positive and gram-negative [29-31]. Unique photocatalytic activity of TiO_2 can lead to higher reactive oxygen species this might cause oxidative stress and breakdown bacterial cell walls, acting as an antibacterial agent [32,33].

To the best of author's knowledge, no prior research has compared these two sealers incorporated with novel antibacterial agent $SiTiO_2$ NPs. Bio-C+2% $SiTiO_2$ showed more significant antimicrobial activity, when compared to other groups followed by Bio-C+1% $SiTiO_2$ group, MTA Fillapex+ 2% $SiTiO_2$ NPs group, Bio-C, MTA Fillapex+1% $SiTiO_2$ and least for the MTA group.

Limitation(s)

In the present study, the maximum incubation period was only seven days, and longer periods of antimicrobial effect of these sealers, may be examined in future research. A sealers, that is effective against one microbe in-vitro might not necessarily be effective against the same bacterium in-vivo. Thus, it is necessary to do further research utilising the same medications for longer periods in clinical trials of all infected canals to validate the results.

CONCLUSION(S)

Within the confines of the present study's limitations, it can be concluded that, root canal sealers modified with $SiTiO_2$ NPs shows long term antimicrobial properties in a dose-dependent manner, while maintaining flow compliance with the acceptable ISO standard. $SiTiO_2$ NPs had limited effect on the antimicrobial properties of MTA Fillapex, when compared to the Bio-C sealer. $SiTiO_2$ NPs could potentially reduce bacterial proliferation and the incidence of secondary root canal infections.

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