RESEARCH PAPER

An Analysis of the Physicochemical Properties of Total Fill Bio Ceramic Root Canal Sealer Loaded with Chitosan Nanoparticles

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ABSTRACT

This study aimed to determine the incorporation of Total Fill endodontic bioceramic sealer with the chitosan nanoparticles, where the relationship between the degree of conversion (DC) and antimicrobial effect were assessed. Total Fill bioceramic sealers mixed with chitosan nanoparticles in different concentrations (2.5%,5%,10%). The antibacterial activity against *Enterococcus faecalis* was measured by the direct contact test (DCT) at different time intervals (immediately, 30 min., 24 hours, 48 hours, 7 days). Fourier Transformed Infrared Spectroscopy measures the degree of conversion. The Kruskal–Wallis and Mann-Whitney U tests were used to analyze group differences and a post hoc comparison of Duncan's multiple range test. Pearson correlation test and Linear regression analysis were used to determine the correlation between the degree of conversion and antibacterial activity between groups. The statistical analyses were computed at a 0.05 significance level. There is a significant difference between the readings of antibacterial activity and the degree of conversion at different time intervals (P=0.05). The antibacterial activity increased as the nanoparticle concentrations increased, and the degree of conversion decreased as the chitosan nanoparticles increased. Positive, strong relation between the degree of conversion and antimicrobial activity.

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INTRODUCTION

One of the main reasons for endodontic treatment failure is the remaining bacteria within the complex canal anatomy that is difficult to completely eradicate with cleaning and disinfecting protocols.[1] During the endodontic treatment procedures, the bacterial population is considerably reduced by instrumentation, irrigation, and intracanal medicaments [3]. In addition, an antibacterial effect of root canal filling materials, such as endodontic sealers, is typically used to minimize the concentration of remaining microorganisms in the root canal [4].

The intratubular penetration of the bioceramic sealer could make a micromechanical interlock within the radicular dentine. In addition, the wetness that remains in the dentinal tubules could excite their setting reaction with the production of hydroxyapatite, thus creating the chemical bond

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with the root dentine [5]. The micromechanical interlock and the chemical bond increase the resistance to any filling material separation, increasing the root strength and preventing fractures [6]. This material mostly includes alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates [7].

The classification of bioceramic materials into bioactive or bioinert materials is a function of their interaction with the surrounding living tissue. Bioactive materials, such as glass and calcium phosphate, interact with the surrounding tissue to encourage the growth of more durable tissues [8], whereas Bioinert materials, such as zirconia and alumina, produce a negligible response from the surrounding tissue, effectively having no biological or physiological effect [9]. A study was conducted using different conditions to examine whether the presence of water can affect the antimicrobial properties of this bioceramic sealer, and no significant differences were observed in bacterial survival with or without storage in water. [10].

This evidence indicates that the high value of the initial pH of the endodontic sealers may not be the only factor that explains their antimicrobial activity. On the other hand, the degree of conversion (DC) can be defined as the extent to which monomers react to form polymers or as the ratio of C=C double bonds converted into C-C single bonds. In the polymerization of bifunctional methacrylate, the complete conversion is never attainable because diffusional restrictions in the later stages of the polymerization reaction prevent a certain amount of monomer molecules from reaching reaction sites. Thus, the DC in dental composites usually varies between 50 and 80%. [11].

Fourier Transformed Infrared Spectroscopy (FTIR) analysis of the bioceramic sealer showed that the broad presented peaks at 3350 and 3594cm−1 represent hydroxyl ions' stretching motions in the bioceramic sealer crystal network [12]. The observed peaks at 2935 and 2900 cm-1 and a bent peak at 1476 cm−1 represent the stretching motions of C-H in the alkane group. The peaks ranging from 1733, 1619 and 1607 cm−1 and 1170 cm−1 and 1733 cm−1 are associated with stretching bands of C=O and C-O, respectively. The bent peaks ranging from 828 to 892cm−1 are associated with Si-O-Si stretching vibrations and $Si\text{-}(CH_3)_3$.[13] The DC is directly related to the decline in the absorption band at 3350 and 3594cm-1 on the FTIR spectra as in the following equation: DC % = (A0 – At / A0) x 100. (A0) = absorption peak (area of the band) at 3350 cm-1 when time = 0 (base paste before mixing), (At) = absorption area of the band at different time intervals after sealer mixing. This band area at 3350 cm-1 will be measured using Motic Images Plus 3.0 software for image analysis [14].

Our study will focus on the Total fill bioceramic sealer. Enterococcus faecalis was selected for the antibacterial study since they are the most prevalent persistent pathogen in failed root canal treatment with a protonpump inhibitor mechanism that can withstand a high pH range of 11.5 [2]. The significance of concentrating on Total Fill bioceramic sealer lies to a great extent in two main points; the first is to determine the effect of incorporating the different nanoparticles on the action of the Bioceramic sealer, especially on the antimicrobial activity. The second is to evaluate the effect of incorporating the different nanoparticles on the degree of conversion of the Total Fill bioceramic sealer. The findings and results will update the current knowledge of some advantages of the use of nanoparticles in the field of endodontics.

The importance of focusing on Total Fill bio ceramic sealer primarily resides in two key aspects; the first is to assess the impact of integrating various nanoparticles concentrations into the performance of the bio ceramic sealer, particularly regarding its antimicrobial efficacy. The second objective is to assess the impact of integrating various nanoparticles concentrations on the degree of conversion of bio ceramic sealers. The findings and results will update the current knowledge of some advantages of the use of nanoparticles in the field of endodontics. Thus, it will be an eye-opener for the researcher and every clinician in their daily clinical practice.

Study hypotheses H_o

1. There are no differences in the amount of NP concentration to affect the antimicrobial activity of the bio ceramic sealer.

2. There are no differences in the amount of NP concentration required to affect the bio ceramic sealer's conversion degree.

MATERIALS AND METHODS

The pre-mixed Total Fill Bioceramic sealer (FKG Dentaire/France), which was dispensed using a

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syringe, was used. They were purchased from a UAE brand distributor (Dubai Medical Company). The chitosan nanoparticles were purchased from Sigma Aldrich Company USA and Nano Phi company in Baghdad /Iraq, which are brand distributors. Meanwhile, the chitosan nanoparticles size 50-80 nm (Chengdu Alpha Nano Technology/China) were consumed in three concentrations (2.5 %, 5 %, and 10 %). The sealers and the nanoparticles were weighed on a precision scale (Micronal S/A, model AB 204, São Paulo, SP, Brazil). The process of mixing and incorporation between sealer and nanoparticles was conducted on an unpolished glass plate with roughness average (Ra) 2-5 micrometers (Larident SrL, Italy). The surface's roughness will facilitate the nanomaterial granules' dispersion, promoting better incorporation [13]. The mixing process was continuous and consistent until it reached a homogeneous consistency. The materials will be divided into four main groups based on the chitosan concentrations, followed by five subgroups based on different time intervals.

Fourier Transform Infrared Spectroscopy (FTIR)

A small amount from the set sealer samples from every time intervals were mixed with potassium bromide (KBr)by using mortar and pestle and packed into a special metal container (holder), put on the press, and compressed to 8 tons to achieve a disc shape of 1cm diameter before incubated at 37^{o}C [15]. The DC will be measured at five intervals (immediately after the mixture, 30 minutes, 24 hours, 48 hours, and a week). The freshly mixed samples were applied to a special glass slab, a part of the FTIR machine (FTIR 600 Biotech Engineering Co. Ltd England), in a thin layer for the measurement process.

Antimicrobial activity (Bauer–Kirby Test)

E. faecalis ATCC BAA-2128 was selected following the Development Organization, USA standards. The bacteria were purchased from the Media Microbiology Lab in Erbil/ Iraq. A brain heart infusion of broth agar was inoculated with two isolated colonies of *E. faecalis* and left for 30 minutes. 20mL of Muller–Hinton (MH) agar was aseptically poured into sterile Petri dishes, and a loopful of inoculated brain heart infusion (BHI) broth was spread on the Muller-Hinton agar and incubated aerobically for 48 hours at 37°C. Each group of Total Fill bioceramic sealer was added, which was repeated 10 times for every sample at different intervals) [16]. The samples were applied on a 6 mm (in diameter) filter disc, placed on the surface of inoculated Muller-Hinton media and incubated at 37°c. The bacteria's growth inhibition zone will be measured with a millimeter ruler.

Scanning Electron Microscopy (SEM)

The surface morphology of chitosan nanoparticles (50–80 nm) was analyzed utilizing a scanning electron microscope (JEOL JSM-6510LV, Tokyo, Japan). Samples were affixed on aluminum stubs using carbon glue and subsequently sputter-

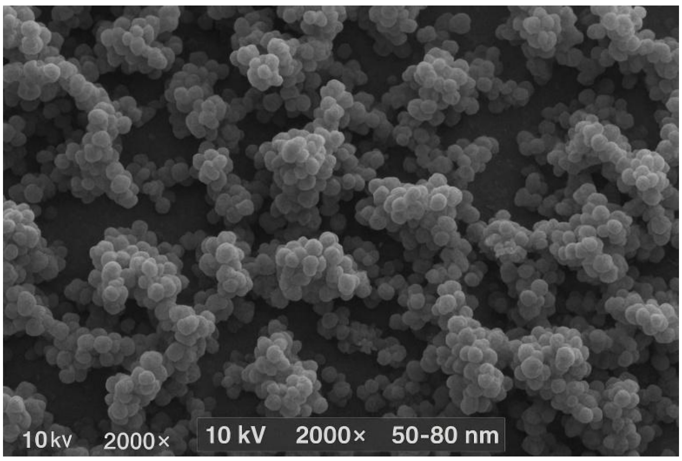

Fig. 1. SEM micrographs of chitosan showing overall surface morphology, irregular and porous structure (2,000×).

coated with a thin (5 nm) layer of gold to enhance conductivity. Micrographs were acquired at an accelerating voltage of 10 kV with a secondary electron detector at magnifications of 2000X.

Statistical analysis

The data of this research were analyzed by using one-way ANOVA and Duncan's multiple tests for degree of conversion. The Kruskal- Wallis test and Mann-Whitney U test for antibacterial activity of Total Fill bioceramic sealer. Pearson Correlation test and Linear Regression test (R) to evaluate the relation between degree of conversion and antibacterial activity of Total Fill bioceramic sealer.

RESULTS AND DISCUSSION

The degree of conversion data of the Total Fill bioceramic sealer enhanced with chitosan nanoparticles were analyzed using the one-way ANOVA Table 1.

This table illustrates the enhanced conversion degree of Total Fill bioceramic sealer modified with chitosan nanoparticles, observed at various time intervals and nanoparticle concentrations. There was a significant difference at P=0.05.

Antibacterial activity Total fill with chitosan % nanoparticles against (E. faecalis)

The data of antibacterial activity of Total Fill bioceramic sealer modified with chitosan nano particles were analyzed by Kruskal Wallis test as shown in Table 2.

In this table we can see the antibacterial activity of Total Fill bioceramic sealer modified with chitosan nanoparticles against E. faecalis was increased at different time intervals and with

Table 1. One-way ANOVA for Degree of Conversion Total Fill with Chitosan nanoparticles.

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	6485.064	4	1621.266	6.652E3	.000
control	Within Groups	10.968	45	.244		
	Total	6496.032	49			
	Between Groups	3833.916	4	958.479	4.543E3	.000
2.5 %	Within Groups	9.494	45	.211		
	Total	3843.410	49			
	Between Groups	3791.829	4	947.957	4.068E3	.000
5 %	Within Groups	10.487	45	.233		
	Total	3802.316	49			
	Between Groups	3050.338	4	762.584	4.559E3	.000
10 %	Within Groups	7.528	45	.167		
	Total	3057.866	49			

* One ANOVA demonstrated a statistically significant difference among the sealers with modified groups. The chitosan nanoparticles diminished the conversion of Total Fill bioceramic sealer, as the chitosan concentration increased, and the degree of conversion decreased.

Table 2. Kruskal Wallis Test shows the antibacterial activity of Total Fill bioceramic sealer with chitosan % against E. faecalis and it was a significant difference at p value = 0.05.

	immediately	30 min	1 day	2 days	7 days
Total fill control croup	6	10.9	14.6	17.4	17.7
chi-square + sig			45.722 **		
Total fill 2.5 % chitosan	6	12.1	13.4	15	17.3
chi-square + sig			47.212**		
Total fill 5 % chitosan	6.6	12.8	14	18.1	19.4
chi-square + sig			46.364 **		
Total fill 10 % chitosan	6.9	15.4	16.8	19.8	20.1
chi-square + sig			44.802 **		

**mean statistically significant at P=0.05

different nano particles concentrations. and it was a significant difference at P=0.05.

Pearson Correlation test

All data for antibacterial activity and degree of conversion for each bioceramic sealer modified with nano particles (chitosan) were analyzed by Pearson correlation test to find the relationship between the antibacterial activity and degree of conversion.

The amounts of Pearson correlation test are (-1, 0, +1) these numbers mean:

-1: negative relationship, 0: no relationship, +1: positive relationship.

Linear Regression test (R)

All data of degree of conversion and antibacterial activity of the bioceramic sealers modified with chitosan nano particles were analyzed by

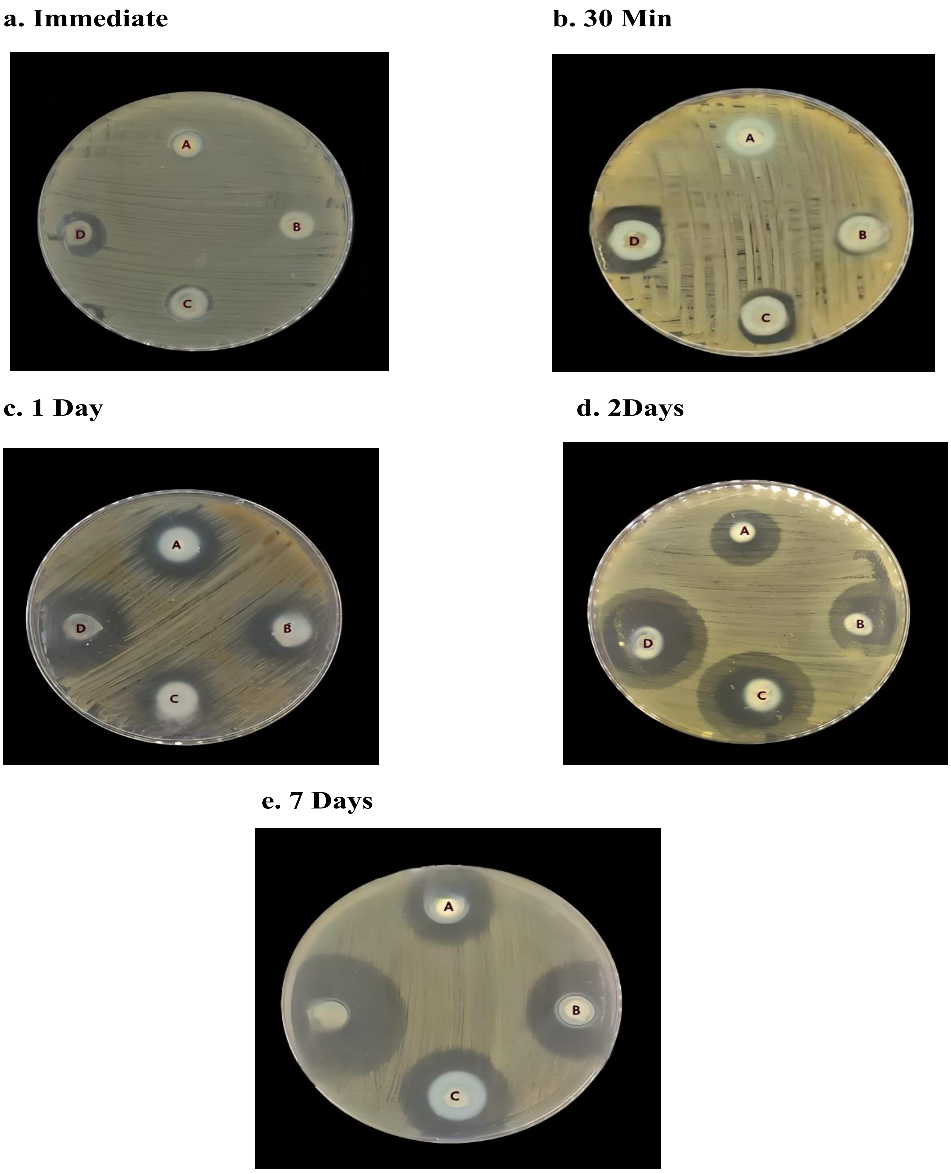

Fig. 2. that contains subfigures (a. immediately, b. 30 Min., c. 1 day, d. 2 days, e. 7 days). show the antibacterial activity of Total Fill bio ceramic sealer modified with chitosan nanoparticles against the E. faecalis at different time intervals.

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linear regression test to find the strength of the correlation between the degree of conversion and antibacterial activity.

The amounts of (R) are (-1, 0, +1)

And according to the results of this research, all readings were above zero, there are further explanations for R amounts as follows:

{0.1 – 0.4} consider a Weak relation between the variables.

{0.5 – 0.7} consider a Moderate relation between the variables.

{0.8 – 1} consider a Strong relation between the variables.

This study's results indicate that the integration of nanoparticles into the bioceramic sealer influenced the degree of conversion. The influence of Chitosan nanoparticles on Total Fill bioceramic sealer results in a reduction of the degree of conversion as the proportion of Chitosan increases over various time intervals, failing to achieve 100% after 7 days. The unmodified Total Fill bioceramic sealer does not achieve a 100% degree of conversion after 7 days; However, the incorporation of chitosan nanoparticles into the sealer (at concentrations of 2.5%, 5%, and 10%) resulted in a reduction greater than that of the original sealer. The kind, size, and concentration of nanoparticles will also affect the degree of conversion of the sealer [17,18].

Silvia C. etal 2016. Evaluated the conversion

Table 3. Demonstrate there is a positive relation between the degree of conversion and the antibacterial activity of Total Fill bioceramic sealer modified with chitosan nanoparticles against E. faecalis.

		Antibacterial activity Total Fill (E. faecalis) chitosan	Degree of Conversion total fill Chitosan
Antibacterial activity Total Fill (E. faecalis) chitosan%	Pearson Correlation	1	.703**
	Sig. (2-tailed)		.000
	N	40	40
Degree of conversion total fill Chitosan%	Pearson Correlation	.703**	1
	Sig. (2-tailed)	.000	
	N	40	40

**Correlation is significant at the 0.01 level (2-tailed).

Table 4. Demonstrate the R value for linear Regression test that explains the strength of the relation between the antibacterial activity of Total Fill against E. faecalis modified with (chitosan%) and degree of conversion of Total Fill modified with Chitosan % shows a strong relation between the variables.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.703[a]	.494	.492	7.05304

a. Predictors: (Constant), VAR00003

Model		Sum of Squares	Mean Square	F	Sig.
1	Regression	9631.913	9631.913	193.624	.000[a]
	Residual	9849.586	49.745		
	Total	19481.498			

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	51.374	1.605		32.004	.000
	VAR00003	1.515	.109	.703	13.915	.000

a. Dependent Variable: VAR00004

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degree of Real Seal SE (RSSE) bioceramic self-cure sealer against Real Seal SE dual-cure sealer at various root levels (apical, middle, and coronal) during different time intervals (24 hours, 48 hours, and 7 days). The degree of conversion (DC) for Real seal bioceramic sealer (self-cure) exceeded that of the dual cure across all time periods at various root levels.[17]

Inaam Baghdadi et al. (2020) conducted experiments on a bioceramic-based root canal sealer aimed at enhancing its physicochemical properties through reinforcement with three distinct nanomaterials: multi-walled carbon nanotubes (MWCNTs), titanium carbide (TC), or boron nitride (BN) at two weight percentages (1 wt% and 2 wt%). The root canal sealer assessed in this study was BioRoot. The one-weight. % composites exhibited markedly reduced beginning and ultimate setting times in comparison to the unaltered BioRoot™ RCS ($p < 0.05$). The 2-wt.% composites demonstrated extended initial setting periods, however markedly reduced ultimate setting times compared to BioRoot RCS [18].

Enterococcus faecalis has been utilized in numerous in vitro studies to evaluate the antibacterial efficacy of endodontic sealers and their efficiency in root canal therapy. Consequently, these microorganisms are employed in the present investigation to assess the antibacterial efficacy of the evaluated Total Fill bioceramic sealer. The discrepancies may primarily stem from variations in microorganism strains utilized, time intervals, testing methodologies (DCT or ADT), and bacterial resistance [19]. Incubation methods (aerobic or anaerobic) [20]. Types of agar media (selective or non-selective), types of testing materials (bioceramic sealer), and the number of bacterial colonies. Additionally, there may be factors contributing to the discrepancies between our findings and those of other studies [21]. The kind, size, and concentration of nanoparticles will also affect the antibacterial capabilities of the sealer [22].

Iqbal et al. (2023) discovered that the integration of specific types and quantities of nanoparticles into endodontic sealers exhibited antibacterial properties in vitro. The necessity for well-designed clinical research to translate in vitro findings into clinical practice is essential. The integration of nanoparticles may augment the antibacterial efficacy of endodontic sealers and boost treatment results [22].

Carvalho, N. K. Et al. (2021) demonstrated that the integration of chlorhexidine-hexametaphosphate nanoparticles (CHX-HMP NPs) enhanced the antibacterial efficacy, as evidenced by the Direct Contact Test. This enhancement was observed in AH Plus bioceramic sealer, pulp canal sealer (PCS), and MTA bioceramic sealer. The sealers exhibited a reduction in flow and an increase in solubility after 24 hours of immersion, yet did not influence the radiopacity of the samples. The setting time of AH plus increased, while MTA failed to achieve setting under any of the tested conditions. All samples exhibited a reduction in pH value as immersion time increased [23].

CONCLUSION

This research indicates that root canal bioceramic sealers combined with 2.5%, 5%, and 10% Chitosan nanoparticles exhibited enhanced antibacterial efficacy compared to the sealer used alone. The incorporation of nanoparticles with endodontic sealers offers enhanced benefits by controlling and minimizing post-endodontic treatment infections, which is also advantageous in retreatment scenarios. There are many points we can conclude from this research:

1. The degree of conversion of Total Fill bioceramic sealers were affected by the addition of nanoparticles (chitosan) with different concentrations at different time intervals.
2. As chitosan nanoparticles concentrations increased the degree of conversion decreased in Total Fill bioceramic sealer.
3. The antibacterial activity of Total Fill bioceramic sealer loaded with (2.5% ,5% ,10%) chitosan nanoparticles against E. faecalis increased as the concentration of NPs increased at different time intervals.
4. Strong positive relation between degree of conversion and antibacterial activity of Total Fill bioceramic sealer.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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