

## ANTIMICROBIAL ACTIVITY, PHYSICAL PROPERTIES AND SEALING ABILITY OF EPOXY RESIN-BASED SEALER IMPREGNATED WITH GREEN TEA EXTRACT-CHITOSAN MICROCAPSULES: AN IN-VITRO STUDY

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### ABSTRACT

**Aim:** To assess the effect of prepared green tea-chitosan microcapsules on the antibacterial efficiency against *Enterococcus faecalis*, the physical properties, and the sealing ability of resin-based endodontic sealer impregnated with these microcapsules.

**Materials and Methods:** Green tea extract (GTE) and chitosan solution were prepared, then GTE was dissolved into this solution followed by precipitation of chitosan nanoparticles (CSNPs) loaded with GTE. Microcapsulation of GTE-CSNPs was performed and the solution was prepared (3 % w/v concentration). Characterization of microcapsules was conducted using Fourier-Transform Infrared Spectroscopy (FTIR), Zeta potential, and transmission electron microscope (TEM). The release of GTE at different pH values was evaluated. GTE-CSNPs solution was added to an epoxy resin-based sealer and the antibacterial activity was evaluated via direct contact test. The physical properties of the modified sealer were evaluated according to American Dental Association (ADA) specifications. Sealing ability was evaluated stereomicroscopically. Data were statistically analyzed.

**Results:** FTIR and Zeta potential ensured the attachment between GTE and Chitosan. The particle size of CSNPs and GTE-CSNPs was around 20 nm, while microcapsules were greater than 1000 nm. A significantly higher percentage of GTE was released at pH 5 than pH 7.4, ( $P < 0.001$ ). After sealer modification, the antibacterial evaluation showed significant decrease in optical density ( $P < 0.001$ ). Also, the setting time, film thickness and microleakage were significantly decreased, however, the solubility was significantly increased ( $P < 0.001$ ) but still within the acceptable limit of ADA specifications.

**Conclusions:** GTE-Chitosan microcapsule is a proper natural candidate for improvement of sealer's properties.

**KEYWORDS:** Antimicrobial activity, Chitosan, Endodontic sealer, Green tea, Sealing Ability.

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## INTRODUCTION

Endodontic sealers are used for root canal obturation to provide a hermetic seal along the canal and prevent reinfection. To decrease the bacterial population, many attempts have been made to improve the antimicrobial activity of endodontic sealers via the addition of antibiotics<sup>1,2</sup> or antimicrobial nanoparticles.<sup>3</sup> Recently, natural products have attracted great attention in the medical field, as alternatives to synthetic chemicals, based on their low toxicity, lack of drug resistance, and cost-effectiveness.<sup>4</sup> Various herbal alternatives such as Neem, Garlic, green tea polyphenols, etc. proved their efficient antimicrobial property in different dental applications.

Green tea is one of the most widely consumed health beverages obtained from *Camellia sinensis*. It contains many active components, mainly polyphenols that are characterized by their antioxidant, anticarcinogenic, anti-inflammatory, and antibacterial properties.<sup>5</sup> To allow sustained release of this natural antimicrobial agent and get benefits of its medicinal properties, it needs to be encapsulated or used in conjunction with a drug carrier. Several studies have evaluated the use of chitosan, a natural polysaccharide, as a drug carriers' system and considered it a successful polymer for such application.<sup>6,7</sup> Chitosan is characterized by its biocompatibility, biodegradability, and mucoadhesivity.<sup>5,8</sup>

Most of the researches in endodontics were conducted to evaluate the antimicrobial properties of green tea and compare it with other synthetic chemicals. However, scarce information is available regarding the effect of this natural product on the antimicrobial and physical properties of endodontic sealers. Therefore, the current study aimed to prepare and characterize green tea-chitosan microcapsules as well as to evaluate its antibacterial efficiency against *Enterococcus faecalis*, the alteration of physical properties, and the sealing ability of resin-based endodontic sealer impregnated with these microcapsules.

The null hypothesis was that there is a non-significant difference between the modified sealer with green tea extract-chitosan microcapsules and the unmodified one in terms of antimicrobial activity and the properties to be tested.

## MATERIAL AND METHODS

### Preparation of Green Tea Extract (GTE)

Dried green tea leaves (*Camellia sinensis*, 100 g, Japanese origin) were powdered in a Warring blender and extracted with double distilled water (1 L) at 85 °C for 1 h. The extract was filtered through a nylon filter, and the filtrate was centrifuged at 9000 rpm for 15 min. The clear supernatant was removed, and the residual pellet was shaken with distilled water, warmed at 35°C, and centrifuged again. The supernatants were pooled, lyophilized, and the resulting material was stored at -20°C in a screw-capped bottle.

### Preparation of GTE-Chitosan microcapsules

Chitosan (CS) solution was firstly prepared by dissolving 1 g of chitosan powder (low molecular weight, DD85%, Loba Chemi, India) in 200 mL 1% acetic acid (pH = 4). The mixture was stirred for 6 h to get a homogenous solution.

The prepared GTE was dissolved in the chitosan solution to get the final concentration of 30% w/w. Then, 150 mL of tripolyphosphate (TPP) (0.2% w/v) was slowly added dropwise into the solution under magnetic stirring for 20 min. to precipitate chitosan nanoparticles (CSNPs) with GTE following the ionotropic gelation process.<sup>9</sup> Afterward, this solution, which contains CSNPs loaded with GTE and remnant of the original solution, was added dropwise into sodium hydroxide 1 mol solution to precipitate the remnant chitosan as microparticles. These precipitated microparticles surround GTE-CSNPs forming microcapsules as illustrated in **Figure 1**. The excess sodium hydroxide was washed away. Finally, stock solution in concentration 3% w/v (CS:

GTE = 70:30 w/w) was obtained. For characterization, 0.5 g of pure chitosan nanoparticles were prepared according to the ionotropic gelation process.<sup>9</sup>

### Materials Characterization

The structural features of GTE, CSNPs, and GTE-CSNPs microcapsules were estimated by Fourier-Transform Infrared Spectroscopy (FTIR, Vetex70 RAM II, Germany) using KBr discs. Zeta potential was determined by Zetasizer Nano ZS (Malvern Instrumentation Co, Westborough, MA). Also, the morphology and particle size measurements of CSNPs, GTE-CSNPs, and GTE-CSNPs microcapsules were determined by transmission electron microscope (TEM) (JEOL 100 CX, Japan).

### In-Vitro Release Profile Test

The in-vitro cumulative release pattern of GTE from the chitosan microcapsules was carried out up to 96 h in different buffer solutions with different pH; PBS (pH 7.4) and acetate solution (pH=5) (n=5 specimens/group). Briefly, 2 g of GTE-CSNPs microcapsules (3% w/v) solution was incubated while shaking at 120 rpm in 100 mL of buffer solutions at 37°C. Then, 3 mL aliquots were removed from the buffer solutions to be analyzed by UV-Vis spectrophotometry (Evolution™ 300 UV-Vis spectrophotometer, Thermo Scientific, USA) at a wavelength ( $\lambda_{\max}$ ) 271 nm. The withdrawn amount

was replaced with an equal volume of fresh buffer solution to keep the volume constant.

The prepared GTE-CSNPs solution was added to an epoxy resin-based sealer (Adseal, ADS1906071, META Biomed Co.LTD, Chungcheonbuk-do, Korea) to assess its effect on the antibacterial activity against *Enterococcus faecalis*.

### Assessment of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

An overnight culture of *E. faecalis* (ATCC 2367), grown on Mueller-Hinton agar plates, was harvested in brain-heart infusion (BHI) broth and the concentration was adjusted to an optical density of 0.11 at 570 nm. The serial dilution method was performed to assess the MIC.<sup>4</sup> The microbial growth was determined by the presence or absence of turbidity after 24 and 48 h of incubation. The lowest dilution inhibiting the bacterial growth was considered as MIC. Moreover, a loopful of the broth dilutions was taken and streaked on blood agar plates. The growth of bacteria was checked after incubation for 48 h at 37°C. The lowest dilution which showed no growth of bacteria was taken as MBC. Bacterial suspension added to Mueller-Hinton broth without the addition of GTE-CSNPs solution served as the positive control and distilled water was taken as the negative control.<sup>4</sup>

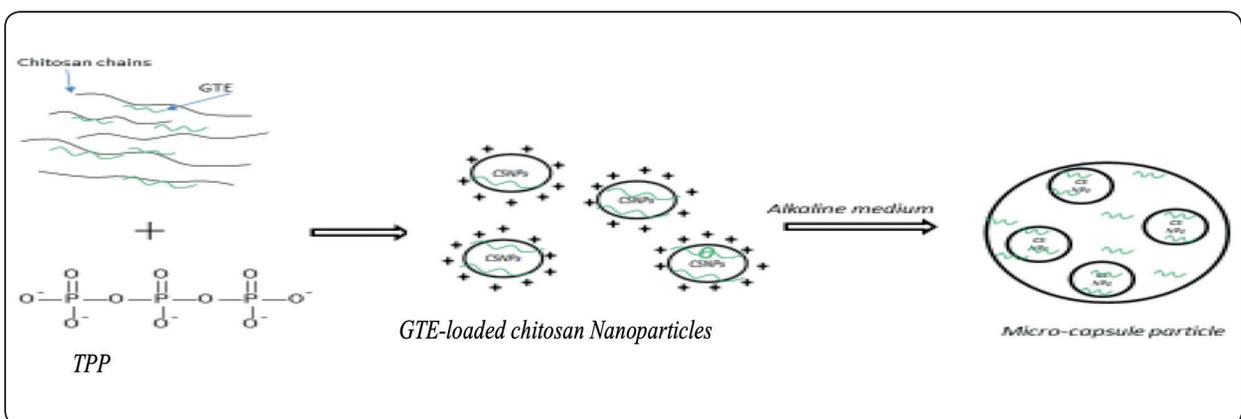


Fig. (1) A diagrammatic illustration of GTE- CSNPs microcapsules formation.

### Antibacterial Activity Assessment

The antibacterial activity against *E. faecalis* was conducted using the direct contact test.<sup>10</sup> The GTE-CSNPs solution was added to the sealer at different concentrations; 25%, 50%, 75%, and 100%, based on MIC and MBC assessment. The unmodified sealer pastes served as control specimens. The bacterial growth kinetics in 96-well microtiter plates was monitored at 620 nm at 37°C using a temperature-controlled microplate spectrophotometer (ELISA Reader, Thermolabsystems, Multiskan Technology, Inc., Finland). A bacterial suspension without the sealer served as the positive control, while BHI media only served as the negative control. The readings were recorded, in optical density (OD) units, every 1 h up to 6 h then at 24 h and 48 h. The experiment was repeated three times to ensure reproducibility.<sup>10</sup> Based on the results of the direct contact test, 100% GTE-CSNPs solution mixed with the sealer paste was selected to investigate its effect on the sealer's physical properties and sealing ability, whereas the unmodified sealer served as a control group. The physical properties of the sealer were examined according to modified ISO 6876/2001 standards<sup>11</sup> and ANSI/ADA's specifications number 57.<sup>12</sup>

### Setting Time

Five stainless steel rings (10 mm inner diameter x 2 mm thickness) were used for each group. The ring was fixed on a glass plate, filled with the sealer, and stored in an incubator at 37°C. A Gilmore needle with a weight of 110 g and an active tip of 1.0 mm diameter was used. The needle was lowered vertically on the sealer surface and the setting time was calculated as the point when the needle failed to make an indentation.<sup>13</sup>

### Film Thickness

Two 5 mm thick glass plates were used (5 samples/group), and their thickness was measured using a digital caliper (Globaltronics, GmbF&Co KG). A total of 0.1g of the sealer was placed in the middle of

one glass plate, then the other plate was positioned centrally to the sealer. Three minutes after the start of mixing, a load of 150 N was applied vertically on top of the glass plate. After 10 min., the load was removed and the thickness of the two glass plates with the sealer in-between was re-measured. The difference of thickness between the two glass plates with and without the sealer was taken as a measure for film thickness.<sup>13</sup>

### Solubility

Five disc-shaped specimens were prepared for each group using a split Teflon mold (20 mm inner diameter, 1.5 mm thickness). The molds, supported by a glass plate covered by celluloid paper, were filled with the sealers. A convenient length of dental floss was inserted into the material before setting to facilitate its handling, and another glass plate covered the mold.<sup>13</sup> The whole assembly was incubated at 37°C, 95% relative humidity for a period corresponding to three times the setting time. The sealers were removed from the mold and weighed three times each with an accuracy of 0.0001g (Sartorius AG, BL210S, Germany) and the mean weight was recorded as W0. The samples were suspended by the dental floss into a glass beaker containing 10 mL distilled water and incubated for 24 h. Afterward, samples were rinsed with distilled water, blotted dry with absorbent paper, placed in desiccators for 24 h, and then reweighed (Wf). The amount of solubility (%) was calculated by the following equation:

$$\text{Solubility\%} = \frac{W0 - Wf}{W0} \times 100$$

### Microleakage Evaluation

Forty single-rooted human mandibular premolar teeth extracted for orthodontic or periodontal reasons were selected for this study and after taking the approval of the Ethics committee [Code no. PD-P-020-007] (10 teeth/gp). Teeth were examined

stereo microscopically for any canal calcifications, caries, cracks, and internal or external resorption and radiographically to ensure a single canal, then, cleaned and stored in distilled water. The teeth were decoronated using a diamond disc to attain a root length of 14 mm.

Teeth were randomly allocated into four groups using a Microsoft Excel random generator software.

- **Group 1:** Obturation with gutta-percha + sealer (control)
- **Group 2:** Obturation with gutta-percha + sealer + 100% GTE-CSNPs
- **Group 3:** Positive control (gutta-percha without sealer)
- **Group 4:** Negative control (teeth with no filled root canal).

For standardization, the whole endodontic procedures were performed by a single operator. All roots were prepared using Revo-S rotary files (Micro-Mega, Besancon, France) up to master apical size 40 at 300 rpm torque 2.2 N/cm. Between every used file, each canal was irrigated with 2 mL of 2.5% NaOCL (Clorox, Egypt) followed by 2 mL of 17 % EDTA (META Biomed Co, Korea). Then, each canal was flushed with 2 mL sterile saline solution. Paper points were used to dry the root canals and finally standardized gutta-percha points (META Biomed Co, Korea) size 40 were used to complete the obturation according to the assigned group with cold lateral condensation technique. All access cavities were sealed using a light-cured composite. A postoperative radiograph was captured to check and ensure proper obturation.<sup>14</sup> All teeth were stored in saline solution at 37°C for 48 h to accomplish the complete setting of the sealer. Then, teeth were thoroughly dried and the roots of all the experimental groups and the positive control group were coated twice with nail varnish except for the apical 2mm. While the entire root surface and apical foramen of the negative control group were

completely coated with the nail varnish. The teeth were allowed to dry completely, then placed in 2% methylene blue dye solution (Loba Chimie, India) at 37°C for two weeks.<sup>15</sup> Afterward, the teeth were rinsed under tap water to remove the dye away from the outer roots' surfaces.

Each root was sectioned longitudinally using a diamond disc, and each half was analyzed under a stereomicroscope with an ocular micrometer (Leica MZ16FA, Leica, Wetzlar, Germany). The extent of dye penetration was measured to assess the microleakage.

### Statistical Analysis

One-way ANOVA test and post-hoc comparisons (Bonferroni and Tukey tests) were used to explore the effect of different pH and time on GTE release, bacterial optical density, and the effect of materials on microleakage. The Independent sample t-test was used to explore the effect of material on physical properties. Results were analyzed using SPSS (statistical package for social sciences, IBM SPSS Statistics for Mac, version 24 software, Armonk, NY: IBM Corp, USA). Data were presented as means and standard deviation. The significance level was set at  $P \leq 0.05$ .

## RESULTS

### Materials Characterization

The FTIR of GTE, CSNPs, and GTE-CSNPs are identical to each other because of the similarity of functional groups for both CSNPs and GTE (**Figure 2**). In Chitosan-TPP nanoparticles, the characteristic absorption peak at 3360  $\text{cm}^{-1}$  was assigned to stretching vibration mode of N-H overlapped with O-H stretching vibration mode. The peak at 2870  $\text{cm}^{-1}$  is due to C-H stretching vibration mode, the 1630  $\text{cm}^{-1}$  peak of -NH bending vibration and peak at position 1530  $\text{cm}^{-1}$  which assigned to N-O stretching vibration, indicating that the tripolyphosphate anions were cross-linked with ammonium groups of chitosan to form CSNPs.

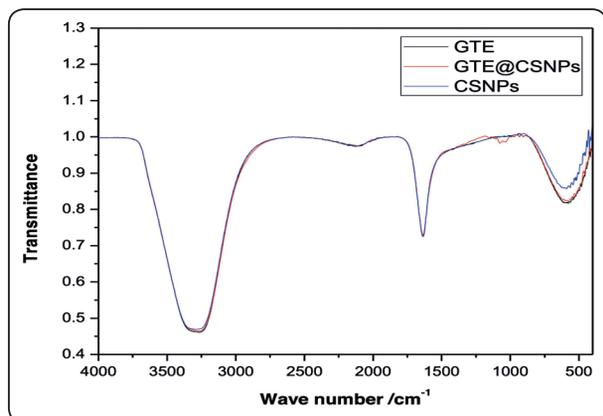


Fig. (2) FT-IR spectra of CSNP, GTE and GTE-CSNPs.

The results also showed that the zeta potential of GTE was -21.5 mV while CSNPs were +37.9 mV. The incorporation of the GTE has intensively decreased (one-fifth) the zeta potential as compared to the corresponding plain CSNPs from +37.9 to +7.95 mV confirming the attachment between GTE

and chitosan (**Figure 3**). Regarding the morphology and particle size analysis, the transmission electron micrographs revealed a spherical shape of CSNPs and GTE-CSNPs and GTE-CSNPs microcapsules. No significant change in size was observed before or after the addition of GTE to CSNPs (both around 20 nm), while the size increased dramatically to be greater than 1000 nm in GTE-CSNPs microcapsules (**Figure 4**).

### Release Profile of GTE

The cumulative release pattern of the loaded GTE from the GTE-CSNPs microcapsules at different pH is shown in **Table (1)** and illustrated in **Figure 5**. Results showed that the percent of GTE released at pH 5 increased gradually with time until attaining an almost constant value; 74% after 8 h. While at pH 7.4, the most cumulative release of GTE was

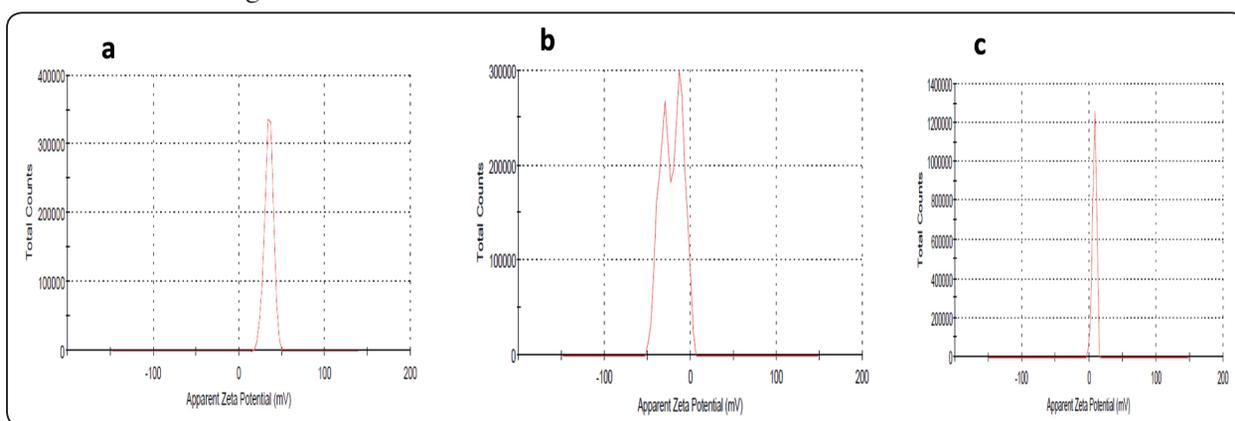


Fig. (3) The zeta potential distribution of (a): CSNPs, (b): GTE and (c): GTE-CSNPs.

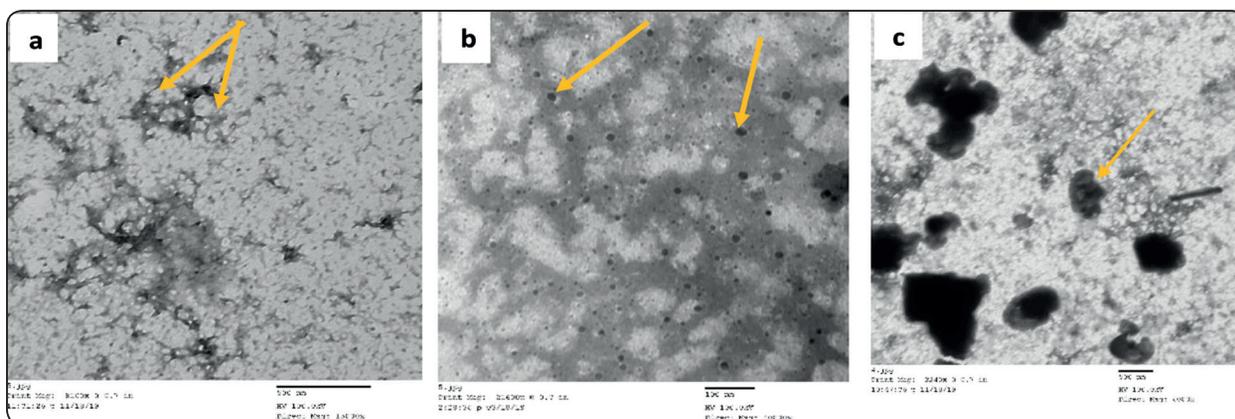


Fig. (4) Transmission electron micrographs of (a): CSNPs, (b): GTE-CSNPs and (c): GTE-CSNPs microcapsules.

18-20% attained after the same time (8 h). The data also demonstrated a significantly higher percentage of the GTE release at pH 5 as compared to pH 7.4 at all-time intervals ( $P < 0.001$ ).

### Results of MIC and MBC

No turbidity was observed at 25% concentration of GTE-CSNPs solution; thus, this was considered as the MIC of the solution. However, by incubation of blood agar plates, bacterial growth was noticed at 25% conc., but no growth was noticed at 50%, 75%, and 100% conc. Therefore, 50% concentration of GTE-CSNPs solution was considered as the MBC of the solution. As expected, positive control showed bacterial growth which was absent in negative control.

### Direct Contact Test

Results of the direct contact test are shown in **Table (2)** and **Figure 6**. Results showed that the addition of GTE-CSNPs solution to the sealer resulted in a significant decrease in the OD at all the investigated concentrations with the highest inhibition of the bacterial growth recorded at 100% concentration ( $P < 0.001$ ). Moreover, the 100% concentration showed constant and continuous inhibition of the bacterial growth throughout the investigated periods.

### Results of Physical Properties

Means and standard deviations of the measured physical properties are shown in **Table (3)**.

TABLE (1): Mean  $\pm$  standard deviation values of the cumulative release (%) of GTE from CSNPs microcapsules

	15 min.	30 min.	1 h	2 h	8 h	10 h	12 h	24 h	72 h	96 h
<b>pH 5</b>	19.00 $\pm 1.49^{aE}$	22.26 $\pm 0.87^{aD}$	40.25 $\pm 1.47^{aC}$	48.67 $\pm 2.74^{aB}$	73.3 $\pm 5.17^{aA}$	74 $\pm 1.85^{aA}$	74.8 $\pm 4.28^{aA}$	76.3 $\pm 1.06^{aA}$	76.2 $\pm 3.16^{aA}$	75.8 $\pm 2.97^{aA}$
<b>pH 7.4</b>	12.50 $\pm 1.2^{bF}$	15.6 $\pm 0.95^{bE}$	17.2 $\pm 1.58^{bDE}$	17.3 $\pm 1.85^{bDE}$	18 $\pm 1.41^{bCD}$	18.3 $\pm 1.08^{bBCD}$	18.8 $\pm 1.83^{bBC}$	20.1 $\pm 2.16^{bAB}$	20.1 $\pm 2.44^{bAB}$	20.3 $\pm 1.97^{bA}$

*Different small letters indicate significant difference within the same column for every time point. Different capital letters indicate significant difference within the same row for every pH value. ( $P < 0.001$ )*

TABLE (2): Mean  $\pm$  standard deviation values of the optical density (bacterial kinetics) of the GTE-CSNPs solutions added to the sealer at different concentrations

	Immediate	3 h	6 h	24 h	48 h	P-value
<b>Bacterial suspension</b>	0.050 $\pm$ 0.005 <sup>dC</sup>	0.697 $\pm$ 0.0291 <sup>aB</sup>	0.699 $\pm$ 0.0295 <sup>aB</sup>	0.749 $\pm$ 0.06 <sup>aAB</sup>	0.766 $\pm$ 0.044 <sup>aA</sup>	0.0001
<b>Sealer+0% GTE</b>	0.513 $\pm$ 0.021 <sup>aB</sup>	0.644 $\pm$ 0.103 <sup>aA</sup>	0.516 $\pm$ 0.023 <sup>bB</sup>	0.510 $\pm$ 0.021 <sup>bB</sup>	0.514 $\pm$ 0.022 <sup>bB</sup>	0.005
<b>Sealer+25% GTE</b>	0.093 $\pm$ 0.004 <sup>bA</sup>	0.094 $\pm$ 0.005 <sup>bA</sup>	0.094 $\pm$ 0.003 <sup>cA</sup>	0.101 $\pm$ 0.007 <sup>cA</sup>	0.100 $\pm$ 0.007 <sup>cA</sup>	0.157
<b>Sealer+50% GTE</b>	0.086 $\pm$ 0.003 <sup>bC</sup>	0.087 $\pm$ 0.004 <sup>bC</sup>	0.092 $\pm$ 0.001 <sup>cB</sup>	0.100 $\pm$ 0.004 <sup>cA</sup>	0.100 $\pm$ 0.006 <sup>cAB</sup>	0.0001
<b>Sealer+75% GTE</b>	0.098 $\pm$ 0.001 <sup>bB</sup>	0.092 $\pm$ 0.001 <sup>bC</sup>	0.094 $\pm$ 0.003 <sup>cC</sup>	0.100 $\pm$ 0.002 <sup>cA</sup>	0.100 $\pm$ 0.008 <sup>cABC</sup>	0.0001
<b>Sealer+100% GTE</b>	0.064 $\pm$ 0.005 <sup>cA</sup>	0.065 $\pm$ 0.014 <sup>bA</sup>	0.065 $\pm$ 0.01 <sup>dA</sup>	0.065 $\pm$ 0.003 <sup>dA</sup>	0.065 $\pm$ 0.014 <sup>dA</sup>	0.923
<b>P-value</b>	0.0001	0.0001	0.0001	0.0001	0.0001	

*Different small letters indicate significant difference within the same column for every time period. Different capital letters indicate significant difference within the same row for every concentration.*

Results showed a significant decrease in the setting time and film thickness after the addition of GTE-CSNPs solution to the sealer, while the solubility of the modified sealer was significantly increased in comparison to the control group, ( $P < 0.001$ ).

### Microleakage Results

Microleakage results are summarized in **Table (4)**. The modified antibacterial sealer with GTE-CSNPs solution recorded a statistically significant decrease in the microleakage in comparison to the unmodified one, ( $P < 0.001$ ).

TABLE (3): Mean  $\pm$  standard deviation values of the setting time (min), film thickness ( $\mu\text{m}$ ) and solubility (%) of the tested groups

Group	Setting time (min)	Film thickness( $\mu\text{m}$ )	Solubility (%)
<b>Control</b> (sealer +0% GTE)	230.00 <sup>a</sup> $\pm$ 14.27	41.60 <sup>a</sup> $\pm$ 2.07	0.17 <sup>b</sup> $\pm$ 0.08
<b>Sealer+100% GTE</b>	119.60 <sup>b</sup> $\pm$ 11.67	18.20 <sup>b</sup> $\pm$ 2.39	1.20 <sup>a</sup> $\pm$ 0.24

*Different small letters indicate significant difference within the same column ( $P < 0.001$ ).*

TABLE (4): Mean  $\pm$  standard deviation of microleakage (mm) of the different tested groups.

Group	Microleakage (mm)	P-value
<b>Group 1:</b> Control	3.07 <sup>b</sup> $\pm$ 0.57	0.0001
<b>Group 2:</b> Sealer+100%GTE	2.37 <sup>c</sup> $\pm$ 0.13	
<b>Group 3:</b> Positive control (gutta-percha only)	4.35 <sup>a</sup> $\pm$ 0.10	
<b>Group 4:</b> Negative control (No filling)	0.63 <sup>d</sup> $\pm$ 0.008	

*Different small letters in each column indicate significant difference between groups ( $P < 0.001$ ).*

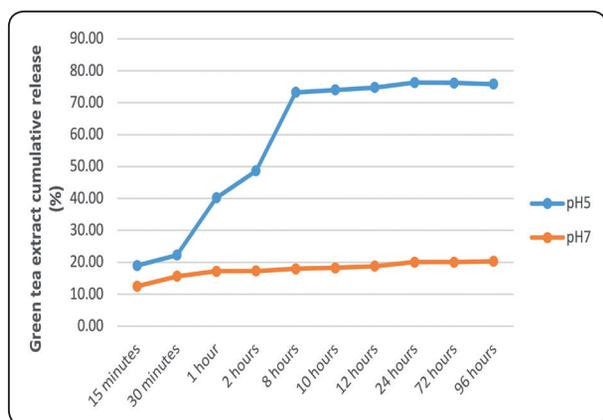


Fig. (5) Cumulative release pattern of the loaded GTE from the microcapsules at different pH values.

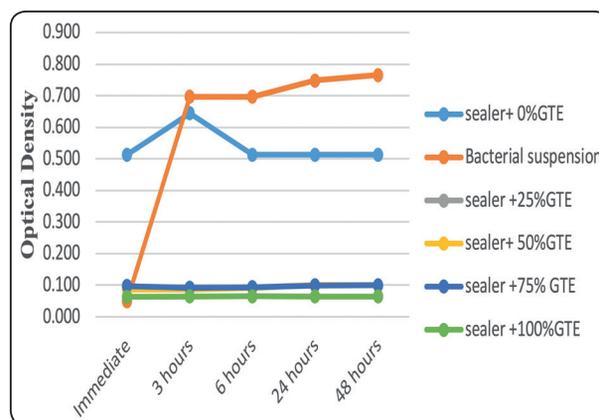


Fig. (6) Mean optical density of the sealer without and with GTE-CSNPs solutions.

## DISCUSSION

The endodontic treatment outcome is greatly affected by the persistence of bacteria after chemico-mechanical disinfection steps. Among the complex microbial environment of the root canal, *E. faecalis*, Gram-positive bacteria, was proved to be the most prevalent bacterium isolated from chronic lesions. This was referred to as its adhesion ability to the root canal dentine, and resistance to disinfection protocols because of biofilms formation that allows the microorganism to survive in the obturated root canals.<sup>4,16</sup>

Generally, all endodontic sealers possess different levels of inherent antibacterial efficiency based on their composition but it's time-dependent, so prevention of root canal re-infection for a long time could not be guaranteed.<sup>17</sup> In the current study, a commonly used epoxy resin sealer; Adseal, was selected, because of the improved biological and physicochemical properties of this sealer's class.<sup>13,18,19</sup>

To improve the antibacterial properties of Adseal, green tea extract was selected as a natural product to be added to the sealer. The GTE has been evaluated in several studies and proved its efficient antibacterial activity as intracanal medicaments in comparison to the commonly used antibiotics and calcium hydroxide.<sup>4,20</sup> At the same time, chitosan nanoparticles were considered as an ideal hydrophilic drug carrier system to carry the GTE and improve its availability because of the high surface area and reactivity of these nanoparticles.<sup>5,8</sup>

In the current study, the chitosan nanoparticles were prepared using the ionotropic gelation method. This method is safe as no heat or organic solvent is used, nor strong shaking is applied so it prevents destruction of any sensitive component and preserves the bioactivity of the existing active compounds.<sup>21</sup> In an attempt to increase the concentration of the loaded GTE to the CSNPs and enhance the sustained release of GTE for a longer

duration, micro-encapsulation of GTE-CSNPs was accomplished.

Our results showed that the cumulative release of the loaded GTE from CSNPs microcapsules was significantly higher at acidic pH than a neutral one, denoting the smart behavior of the microcapsules that augment the GTE release by the presence of infection (**Table 1**). This might be related to the high dissolution of chitosan microcapsule in the acidic media. Moreover, the release pattern of the loaded GTE revealed an initial burst release of about 74% in the first 8 h, which could be related to degradation of microcapsules, due to pH, and release of the GTE from both the microcapsules and nanoparticles. As well, swelling of the hydrophilic polymer probably lead to chain detanglement and formation of pores that allow outward diffusion of GTE and ended up with the polymer dissolution.<sup>6</sup>

A previous study reported a very low inhibitory effect of Adseal against *E. faecalis*.<sup>22</sup> This supported our findings that showed high optical density (OD) of the unmodified sealer group (**Table 2**). However, a significant decrease in the OD was observed after sealer impregnation with GTE-CSNPs, indicating the considerable improvement in the antibacterial activity of Adseal. This is essentially attributed to the dual effect of green tea and chitosan. The proven antimicrobial activity of green tea against *E. faecalis* was explained by the presence of polyphenols<sup>23,24</sup>, mainly the catechins that represent about 60%-80% of the total tea polyphenols.<sup>5</sup> It was found that catechins bind via a hydrogen bonding to the bacterial lipid bilayer structure, causing accumulation of lipid vesicles, leaking the contents that lead to cell membrane expansion, thinning, and ending up with membrane destruction and bacterial death.<sup>4,25</sup> Moreover, chitosan is characterized by a well-known antimicrobial activity related to its cationic nature. Besides, the low MW chitosan permits easy penetration of the bacterial cell walls to bind with DNA leading to inhibition of mRNA synthesis and DNA transcription.<sup>18,26</sup>

Regarding physical properties, our observations revealed a significant decrease in the setting time after the sealer's modification with GTE-CSNPs (**Table 3**). This could be attributed to the additional ring opening polymerization of the epoxy resin that occurs in the presence of low water percent which accelerates the setting reaction.<sup>27,28</sup> The film thickness of the sealer is crucial as it affects the adequate distribution of the sealer inside the root canal. Our results showed a significant decrease in the film thickness of the modified sealer, which might be referred to as the increase in the fluid content; consequently, improved the sealer's flowability. This result was in accordance with the ADA specifications that require film thickness of not more than 50  $\mu\text{m}$ .<sup>12</sup>

Ideally, root canal sealer should be insoluble or display minimal solubility when exposed to periapical fluids to prevent gap formation at the filling/dentin interface and to avoid bacterial ingress which affects the usefulness of the sealer.<sup>29</sup> Our results revealed a significant increase in the sealer's solubility after modification with GTE-CSNPs (**Table 3**), which could be related to the increase in the hydrophilic content of the sealer and leaching out of unreacted particles and loosely bounded fluid components. However, this increased value still falls within the acceptable range (less than 3%) according to the sealer's specifications.<sup>11,12</sup>

One of the major causes for endodontic failure is the microleakage that mostly occurs between the sealer and dentinal walls. Our findings revealed a significant decrease in microleakage after sealer modification with GTE-CSNPs compared to the unmodified one (**Table 4**). This could be explained by the Adseal's expansion that takes place after setting as demonstrated by Lee et al.<sup>30</sup>, improving its sealing ability.

The null hypothesis was rejected as the modified sealer with GTE-CSNPs showed significant improvement in the antibacterial activity, and sealing ability with adequate physical properties in comparison to the unmodified one.

## CONCLUSIONS

The GTE-CSNPs microcapsule is a proper natural candidate for the improvement of antimicrobial activity and sealing ability of epoxy resin-based endodontic sealer. The modified sealer met the requirement for physical properties. Clinical trial study would be beneficial in evaluating the clinical performance of this modified sealer.

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