Assessment of Post-operative Pain and Antibacterial Effectiveness of Chitosan Nanoparticles versus Sodium Hypochlorite Irrigation in Infected Root Canal. (A Randomized Clinical Trial)

Sarah El-Sayed Mahgoub[1], Siza Yacoub Zaghari [2], Shaimaa Gawdat [3], Olfat Shaker [4]

[1] Ph.D. Candidate at the Department of Endodontics, Faculty of Dentistry, Cairo University,

[1] Assistant Lecturer of Endodontics, Faculty of Dentistry, Beni-Suef University

[2]Professor of Endodontics, Faculty of Dentistry, Cairo University

[3]Professor of Endodontics, Faculty of Dentistry, Cairo University

[4]Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University

Corresponding author E-mail: sarah.mahgoub@dentistry.cu.edu.eg

ABSTRACT

Aim: The aim of the study was to assess the bacterial reduction and postoperative pain of Chitosan nanoparticles (3% CNPs) versus Sodium hypochlorite (2.5% NaOCl) when used as a root canal irrigant during instrumentation after single visit endodontic treatment of patients with necrotic maxillary anterior teeth.

Methodology: Twenty two patients with necrotic maxillary anterior teeth, were divided equally into two groups, (n=11) according to the protocol of irrigation; Group A: Irrigation with Chitosan nanoparticles (3% CNPs); Group B: Irrigation with Sodium hypochlorite (2.5% NaOCl). Instrumentation was done using rotary ProTaper files in a crown down manner. During preparation, canals were irrigated using a total of 10 mL of irrigating solutions of the respective test group. After complete preparation, the canals were flushed using 5 ml of the study irrigating solutions. Samples were collected from root canals before preparation (S1) and after irrigation with the experimental solutions (S2), then the root canal culture samples were analyzed by counting the colony forming units (CFUs/ ml). All patients were asked to record their post-operative pain levels using a numerical rate scale (NRS).

Results: There was no statistical significant difference in bacterial percentage reduction between the two groups. (p = 0.176). Regarding postoperative pain incidence and intensity at all follow-up intervals, there was no statistical significant difference between the two groups.

Conclusions: Both of the irrigation protocols had efficient antibacterial effect against *E. faecalis*. Manually agitated chitosan nanoparticles (3% CNPs) can be utilized as an antibacterial irrigant in eradication of *E. faecalis* from root canal system and can be considered as a more safe and efficient alternative for sodium hypochlorite against *E. faecalis*.

Keywords: Nano-chitosan, Sodium hypochlorite, Enterococcus faecalis, Manual agitation, Root canal irrigants.

INTRODUCTION:

Microbial aggregates in the complex radicular space, as well as dentinal tubules close to the canals, might occur from pulp infection. Apical periodontitis develops and persists as a result of these microorganisms and their harmful by products [1]. Clinically, an essential feature of endodontic microorganisms is their ability to produce biofilms. Bacterial biofilm communities are naturally adherent to the tooth surface. Most persistent and chronic bacterial pathosis are caused by bacterial biofilm resistance to antimicrobial irrigants such as sodium hypochlorite (NaOCL) and chlorhexidine (CHX) due to inherited microbiological factors and also, the root canal system's intricate architecture [2].

Enterococcus faecalis, is a facultative gram-positive bacteria. It can invade deeply into the dentinal tubules. It was found that it has the ability to endure severe and harsh surviving conditions, including the presence or absence of oxygen in the pulpal space, high PH, and temperature, and hence can withstand chemico-mechanical preparation and intracanal medicaments. *E.faecalis* resistance mechanism is caused by physiological or structural changes in the bacterial cells [3].

Root canal cleaning and shaping play a critical role in endodontic treatment success by reducing microorganisms. Mechanical root canal debridement and shaping fall short of totally eliminating all microorganisms from the radicular space [4]. As a result, the use of multiple endodontic irrigants and chemicals is required to facilitate cleaning beyond mechanical preparation [5].

Sodium hypochlorite (NaOCL) is the bench mark and most powerful and recommended irrigant because of its broad antimicrobial impact, necrotic tissues and collagen dissolving capabilities, and endotoxins inactivation. However, when introduced into periradicular tissues,

sodium hypochlorite (NaOCL) has been shown to have cytotoxic and irritating properties and is incapable of completely debriding the infected root canals or removing bacterial biofilms [5].

Recently, many antimicrobial agents have been advocated in the nanoscale system to improve their physical and biological properties for better debriding of infected root canals and increasing antibacterial efficiency. Chitosan is one of the nanoscale systems made from natural polymers [6].

Chitosan is a polymer with proven bioactivity made from chitin deacetylation that is employed in bio-medical applications because of its antibacterial qualities, biocompatibility, and capacity to repel ageing for a long time and give an antibacterial effect while disinfecting root canals [7]. The antibacterial impact of chitosan nanoparticles (CNPs) might be attributed to electrostatic attraction with the negatively charged bacterial cell wall, resulting in cell wall permeability changes and cell death [8].

Hence, within the scope of our study, no previous randomized clinical trials have evaluated chitosan nanoparticles as a root canal irrigant. This trial was conducted to assess the efficacy of chitosan nanoparticles (CNPs) as an endodontic irrigant versus sodium hypochlorite (NaOCL) on bacterial reduction against *E.faecalis* and postoperative pain in the infected root canals.

MATERIALS AND METHODS:

Study design:

This was a prospective, 1:1, parallel, double blinded, and randomized controlled trial.

Setting and Recruitment:

The trial protocol was registered on www. Clinicaltrials.gov and was approved by the research ethics committee (REC) no. 181017, Faculty of Dentistry, Cairo University. All trial participants received a full explanation of the trial's nature, goals, advantages, and dangers. A printed consent form that detailed the study's objectives was provided to each patient for signature. Participants were recruited from the outpatient clinic, Department of Endodontics, Faculty of Dentistry, Cairo University.

Sample size calculation:

Based on the findings of a prior study Shingare and Chaugule [9], this power analysis used the percentage reduction in bacterial count as the primary outcome. The effect size was determined

as d = (1.4693). The determined sample size was 18 participants, based on a significance alpha level (α) of 5% and Beta (β) level of (20%) i.e. power = 80%. To account for a dropout rate of roughly 25%, the sample size was raised to a total of 22 subjects (11 subjects each group). The sample size was calculated using G* Power Version 3.1.9.2.

Randomization, allocation concealment, and blinding:

Sequence generation was done using computer-generated random numbers. On (https://www.random.org/), a random sequence was generated that randomly assigned the patients to one of two groups with a 1:1 allocation ratio as follows: Group A: Irrigation with Chitosan nanoparticles (3% CNPs), and Group B: Irrigation with Sodium hypochlorite (2.5% NaOCl). The numbers created randomly were written on little folded opaque papers and then inserted into envelopes according to the allocation sequence obtained from the computer software. Before conducting any procedure. The envelopes were placed in a container (box), and on the day of the experiment, each participant blindly grasped one envelope. Both the participants and the laboratory assessors were blinded to the interventions. The patients were told about the treatment steps (as stated on the consent form) but not which of the irrigants would be used. The laboratory assessors were blinded by not including them in sequence generation or allocation concealment, or by not telling them what type of tested irrigants were used

Preparation and characterization of the nanoparticles:

Preparation:

Chitosan nano particles (CNPs) were prepared according to the ionotropic gelation process [10]. Magnetic stirring was used to dissolve CS (0.5%) in acetic acid (1%) in distilled water at room temperature. The cross-linking agent, poly anion sodium tripolyphosphate TTP, was also dropped into the CS solution, and the PH was adjusted to 4. TPP solutions generated a final CS to TPP mass ratio of 3:1 based on the CS concentration utilized.

Characterization:

Transmission electron microscopy (TEM) was used to check the size and shape of the produced nanoparticles. The resultant suspension showed white spherical particles with an average size less than 50 nm.

Eligibility criteria:

Eligible participants were free from any systemic disease of both genders, aging from 18-45 years with asymptomatic single rooted single canal necrotic anterior teeth with or without apical periodontitis. Exclusion criteria included patients with vital pulps, facial swelling or acute infection or pain on percussion.

Interventions:

Eligible patients were allocated into two equal groups on the basis of the irrigating solutions, according to a random sequence.

- In Group A: Chitosan nanoparticles (3% CNPs) was used for irrigation.
- In Group B: Sodium hypochlorite (2.5% NaOCl) was used for irrigation.

Endodontic protocol:

Demographic information such as the patient's age, gender, address, and phone number, as well as all medical and dental histories, were gathered. All data was recorded in a diagnostic chart. The pain levels were recorded using a numerical rate scale (NRS). Patients were then asked to fill a pain dairy accurately and honestly at 24, 48, and 72 hours after endodontic treatment, then get it back to the operator on time.

The NRS is an 11-point scale with two extremities: "no pain" and "pain as awful as it gets." None (0), Mild (1-3), Moderate (4-6), and Severe (7-10) were used to categorize the pain levels. Participants were asked to choose the score that best reflected their pain level.

Patients were anaesthetized then each tooth was isolated using a rubber dam to maintain an aseptic field. The tooth and surrounding field were disinfected using a 3% hydrogen peroxide and 2.5 % NaOCl before and after coronal access cavity preparation. Working length was determined at first by an apex locator and confirmed by an ordinary periapical radiograph to be adjusted at1 mm shorter of the radiographic apex.

The root canals were mechanically instrumented in a crown-down approach with a ProTaper Universal rotary file system up to size F4 (tip #40). Within mechanical instrumentation, canals were thoroughly irrigated for 1 minute between every two subsequent files with a total of 10 mL of irrigating solutions from the respective test group, sodium hypochlorite (2.5% NaOCL) and chitosan nanoparticles (3% CNPs), utilizing a 27-gauge side-vented needle in a plastic syringe inserted 1 ml shorter than the working length. After complete preparation for both groups, the canals were irrigated for 5 minutes using 5 ml of tested irrigants in a plastic syringe using a 27-gauge side- vented needle

inserted 1 mm short of the working length. Irrigation activation was done by manual dynamic agitation with a well-fitted tapered master cone (#40, taper 6%) pumped up and down in rapid vertical strokes that were short (2-3 ml).

Using #40 paper points, all canals were thoroughly dried (corresponding to the size of the tip of the master apical file F4). Root canals were obturated with a modified single cone approach after drying, utilizing a # 30 spreader to allow space for #25 auxiliary points in the coronal third alongside the well-fitting special F4 ProTaper master cone (#40 taper 6%).

All patients were given postoperative instructions to call the operator if they experienced moderate or severe pain, and they were allowed to take a prescription painkiller (ibuprofen 400 mg). If discomfort persisted, patients were encouraged to contact their dentist and visit the clinic for an emergency intervention, signaling a flare-up (emergency).

Microbiological procedures:

Initial sampling:

Initial pre-treatment root canal culture samples (S1) were collected using a sterile # 25 paper point. To assist the entrance of the sterile absorbent paper points to collect the samples, sterile saline solution was introduced into the canals, followed by the insertion of a #20 K-file to at least 1 mm short of the apex. Each paper point was held in place for at least 60 seconds before being inserted in test tubes with freshly prepared transport medium appropriate for the preservation of facultative bacteria and delivered to Cairo University's Molecular Biology Laboratory for microbiologic processing.

Final sampling:

All root canals were flushed with 10 ml of sterile saline before collecting the final sample S2 using a sterile # 40 paper point in the same way as S1.

Root canal samples were collected and cultivated in serial dilution on heated blood agar plates under anaerobic conditions which inactivates inhibitors of the growth, incubated for 48 hours. The bacterial reduction of the tested irrigants was assessed by counting the colony-forming units per ml (CFU/ml) before and after irrigation (S1 and S2) for each patient.

Statistical methods:

Statistical analysis was performed with IBM® SPSS® [2] Statistics Version 20 for Windows. Quantitative data was presented as mean, median, standard deviation (SD), range (minimum – maximum) and 95% confidence interval for the mean (95% CI) values. Data will be explored for normality by checking the data distribution and using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For parametric data, repeated measures analysis of variance (ANOVA) was used to compare between the groups as well as to study the changes by time within each group. For non-parametric data, the Mann-Whitney U test was used to compare between the groups. Friedman's test was used to study the changes over time within each group. Qualitative data was presented as frequencies and percentages. The chi-square test or Fisher's exact test, when applicable, was used for comparisons related to qualitative variables. The significance level was set at ($P \leq 0.05$).

RESULTS:

The study includes 22 patients out of 30 that were enrolled. The range values of age were (32 - 45). The gender distribution showed 9 males and 13 females. The tooth type distribution showed 9 central incisors, 10 lateral incisors and 3 canine. There was no statistical significant difference in age, gender, and tooth type distribution between the two groups ($P > 0.05$), Table (1). CNPs group showed higher bacterial percentage reduction than NaOCL group, however there was no statistical significant difference in bacterial percentage reduction between the two groups ($P > 0.05$), Table (2). Throughout all follow-up periods (24, 48, and 72 hr), the difference in pain intensity or incidence between the CNPs and NaOCl groups was not statistically significant ($P > 0.05$). The NaOCl group, on the other hand, had the greatest pain intensity scores. Post-operative pain intensity gradually declined at the end of few days, Table (3).

Table (1): General demographic data of the participants included in the trial:

Baseline data	Group A	Group B	P - Value
Age (Median & Range)	38.0 (35 - 45)	38.0 (32 - 45)	0.838
Gender distribution			
Male n (%)	4 (36.4%)	5 (45.5%)	0.665
Female n (%)	7 (63.6%)	6 (54.5%)	
Tooth type distribution			
Central n (%)	5 (45.5%)	4 (36.4%)	0.801
Lateral n (%)	5 (45.5%)	5 (45.5%)	
Canine n (%)	1 (9.1%)	2 (18.2%)	

n: Number per group.
(%): Percentage per group.

Table (2): Mean, SD and range values of post- preparation bacterial count and bacterial percentage reduction in the tested groups by Mann-Whitney U test:

Bacterial reduction data	Group A	Group B	P - Value
Pre-preparation bacterial count CFU/ml (Mean, SD, Range)	($10.0 \times 10^5 \pm 9.4 \times 10^5$) ($2.0 \times 10^5 - 30.0 \times 10^5$)	($8.5 \times 10^5 \pm 9.3 \times 10^5$) ($1.0 \times 10^5 - 30.0 \times 10^5$)	0.653
Post-preparation bacterial count CFU/ml (Mean, SD, Range)	($2.0 \times 10^2 \pm 3.2 \times 10^2$) ($0 - 10.0 \times 10^2$)	($5.9 \times 10^2 \pm 10.0 \times 10^2$) ($0 - 30.0 \times 10^2$)	0.653
Bacterial reduction (%) (Mean, SD, Range)	(100.0%±0.1%) (99.9% –100.0%)	(99.9%±0.2%) (99.5% – 100.0%)	0.176

Table (3): Median and range values of NRS score at different time intervals in the tested groups by Mann-Whitney U test:

Different time intervals		Group A	Group B	P - Value
24 hours	**Median**	0	0	0.077
	Min	0	0	
	Max	2.0	7.0	
48 hours	**Median**	0	0	0.154
	Min	0	0	

	Max	0	6.0	
72 hours	**Median**	0	0	0.152
	Min	0	0	
	Max	0	4.0	

NRS: Numerical rating scale.

DISCUSSION:

The goals of endodontic therapy include complete debridement, disinfection, and eradication of pathogenic microorganisms from the complicated configuration of the root canal system. Bacterial eradication from root canals is problematic due to the incapability of existing approaches to disinfect the root canals [11]. Mechanical preparation in combination with adequate irrigation protocols utilizing antibacterial irrigants are two crucial elements in endodontic therapy. This justifies the continuous search for new irrigation solutions and techniques [12]. Thus, the present trial's aim was to assess the antimicrobial potency and postoperative pain of chitosan nanoparticles (CNPs) as an endodontic irrigant and a new alternative to sodium hypochlorite (NaOCl) for disinfecting necrotic teeth.

In this study, *E. faecalis* was selected on the basis that it is one of the most tolerant microorganisms identified in the root-infected canals, with a greater incidence in root-filled teeth with apical periodontitis after treatment [13]. Due to its ability to survive irrigation, intracanal

medicaments, the harsh root canal environment, and its capability to make a protective bio-film that defends against phagocytosis, antibodies, and antimicrobial agents [14].

NaOCl remains the gold standard of irrigating solutions, so it was chosen to evaluate the antimicrobial efficiency as a control. According to Zou *et al.* [15], the antibacterial action of NaOCL is dependent not only on its concentration but also on other factors such as exposure time. As a result, the concentration of NaOCl used in this current study was 2.5% NaOCl.

In the present study, natural irrigation was used in nano form. As documented in the literature, nano-chitosan particles were introduced as an alternate to NaOCl because of their power to break down bacterial cell membranes, interfere with protein synthesis, and improve the mechanical properties of dentine, as well as their biocompatibility, avoiding the toxic effect of NaOCl and providing higher accuracy and efficiency [16], [17], [18]. 3% chitosan nanoparticles of a size less than 50 nm were used. It was recently revealed that 3% CNPs have a potential bacteriocidal impact as an endodontic irrigant against *E. faecalis*, and the difference found between 2.5% NaOCl and 3% CNPs in the eradication of *E. faecalis* from infected canals was not statistically significant [19]. It was found that 2% of CNPs with an average size of 50±5 nm had powerful antimicrobial efficacy against *E. faecalis* [20].

The maxillary central, lateral incisors, and canines were preferred for our investigation because they have a wide, single-large, and straight canal in which the cleanliness process could be examined in the absence of making any methodological errors [21]. According to Smith *et al.* [22], single-rooted teeth are superior for getting consistent results and avoiding loading of bacterial contaminants from other canals in multi-rooted teeth.

Maxillary anterior teeth were instrumented with the rotary Protaper Universal files to size F4 in a crown-down approach to enhance the root canal cleanliness as it acts as a tank of irrigants for better and earlier entrance into the deeper parts of the canal [23].

The bacteriological procedure was performed as initial samples (S1) were collected before mechanical instrumentation to count the number of colony-forming units before preparation using sterile absorbent paper cones, and final samples (S2) were also collected after the final flush with the tested irrigants. In the current study, the antibacterial effect was assessed by counting the colony formation units (CFUs) declared in log CFU/mL. This method had been chosen because it is

characterized by its simplicity and allows quantification of all major viable cultivable microorganisms from root canal space and can be easily done at the laboratory [24], [25].

The intensity of post-operative pain was measured using a numerical rate scale (NRS) in this study. The NRS was found to be straightforward to administer and record, and being more sensitive than the verbal rating scale (VRS) and simpler than the visual analogue scale (VAS) [26], [27]. Some clinical trials showed that postoperative pain improved dramatically during the first 48 hours after endodontic therapy. Therefore, they limited their assessment of postoperative pain to the first 48–72 hours after treatment [28], [29], [30], [31], [32]. On these grounds, pain intensity was recorded postoperatively after 24, 48, and 72 hours.

In terms of the antimicrobial efficiency of the tested irrigants, the difference found between 2.5% NaOCl and 3% CNPs in the eradication of *E. faecalis* from infected canals was not statistically significant. Our results came in agreement with Shrestha *et al*. [33], Del Carpio-Perochena *et al.* [2], Jaiswal *et al.* [34], Roshdy *et al.* [19], and Moukarab [20]. However, our findings were not the same as those of Hassan *et al.* [35]. Results disagreement may be due to the different study circumstances starting with different concentration of nano-chitosan (2%), a discrepancy in the tested strains, variances in methodology and may be different incubation conditions.

Numerous speculations have been proposed to clarify chitosan's antimicrobial action. One proposed theory is that its polycationic nature reacts with the negatively charged microbial cell membrane, modifying cell penetrability and causing hydrolysis of the peptidoglycan on the bacterial cell wall, giving rise to the seepage of proteinaceous and intracellular cytoplasmic contents, suppressing microbial growth and cell lysis [36].

A second proposed hypothesis is based on the sailing mechanism. Sailor agents can chelate metallic ions including calcium, magnesium, and iron, which helps to preserve the biofilm matrix's integrity while also suppressing bacteria growth by lowering enzyme activity. Furthermore, chitosan has the ability to infiltrate bacterial cells and bind to microbial DNA, interfering with mRNA and protein production as well as inhibiting deterioration of bacterial enzymes, thus minimising the risk of bacterial invasion and dentine microfractures [37].

NaOCl stays within the paradigm of root canal irrigants and gains its antimicrobial activity through two mechanisms. First, the chlorine release, which affects a broad range of microbes along

with its ability to dissolve organic debris due to its proteolytic effect, together with the release of oxygen, eradicates anaerobic bacteria and releases more antimicrobial chloramines. Furthermore, hydroxyl ions destroy the lipid membranes of bacteria and DNA, and elevated pH levels cause bacterial protein denaturation [38], [39].

Regarding the intensity of postoperative pain, in the first 24 hours after treatment, the highest level of postoperative pain was documented. Within 48 hours, pain levels in the nano-chitosan and sodium hypochlorite groups had dropped to 0% and 18.2%, respectively. In both groups, the majority of patients reported no pain (median=0) at 72 hours post-operatively. This results in accordance with Alonso-Ezpeleta *et al.* [40], who stated that post-operative pain intensity gradually declined at the end of few days, dropping to 50% at first day and fewer than 10% at seven days.

Throughout all follow-up periods, the difference in pain intensity or incidence between the nano-chitosan and NaOCl groups was not statistically significant. The NaOCl group, on the other hand, had the greatest pain intensity scores.

Chitosan is a natural polysaccharide that is safe for consumption, biocompatible, and not toxic to tissues. This justifies the association of nano-chitosan with lower postoperative pain levels [41], (42). Furthermore, chitosan nanoparticles were considerably less cytotoxic than free soluble chitosan, which was attributed to the presence of a cross-linker in the chitosan nanoparticles, which is anticipated to decrease the charged density on the chains of the chitosan particles and contribute to the less cytotoxic effect [43]. Chitosan's cytotoxicity is proportional to its positive charged density [44], [45].

Amongst the most prevalent causes of post-operative discomfort is the extruding material during root canal preparation and obturation [46]. Apical extrusion of irrigants could be a problem with all preparation techniques and file systems. Hence, the inflammatory reaction could be affected by the type of irrigation solution extruded. This finding could explain the prevalence of lower values of pain intensity in the 3% nano-chitosan group in our investigation.

CONCLUSIONS:

Within the limitations of this study, it can be concluded that:

- Both of the irrigation protocols had an efficient antibacterial effect against *E. faecalis*.

- Manually agitated chitosan nanoparticles (3% CNPs) can be used as an antibacterial irrigant in the eradication of *E. faecalis* from root canal systems and can be considered as a more safe and efficient alternative to sodium hypochlorite against *E. faecalis*.
- Chitosan nanoparticles (3% CNPs) were associated with smaller postoperative pain values compared to sodium hypochlorite (2.5% NaOCl).
- Postoperative pain levels were greatest in the first 24 hours and subsequently reduced overtime, subsiding completely after 3 days.

DISCLOSURE STATEMENT:

No potential conflict of interest was reported by the authors.

AUTHORSHIP STATEMENT:

We confirm that all listed authors meet the authorship criteria and that all authors agree with the manuscript's content.

FUNDING:

This research didn't receive any external funding.

REFERENCES:

(1) Schafer, E., Dent, M., Bossmann, K., Nat, R. (2005): Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against Enterococcus faecalis. *J Endod*, 31 (1): 53-65.

(2) Del Carpio-Perochena, A., Bramante, C.M., Duarte, M.A.H., de Moura, M.R., Aouada, F.A., Kishen, A. (2015): Chelating and Antibacterial Properties of Chitosan Nanoparticles on Dentin. *Restor Dent Endod*, 40 (3): 195-201.

(3) Delgado, J.R.R., Gasparato, H.T., Sipert, R.C., Pinheiro, R.C., Moraes, G.I., Garcia, B.R., Bramante, M.C., Campanelli, P.A., Bernardineli, N. (2010): Antimicrobial effects of calcium hydroxide and chlorhexidine on enterococcus faecalis. *J Endod*, 36 (8): 1309-1393.

(4) Shrestha, A., Cordova, M., Kishen, A. (2015): Photoactivated Polycationic Bioactive Chitosan Nanoparticles Inactivate Bacterial Endotoxins. *J Endod*, 41 (5): 686–91.

(5) Suzuki, S., Masuda, Y., Morisaki, H., Yamada, Y., Kuwata, H., Miyazaki, T. (2014): The Study of Chitosan-Citrate Solution as a Root Canal Irrigant: A Preliminary Report. *Oral Hyg Health*, 2 (4): 142-45.

(6) Akncbay, H., Senel, S., Ay, Z.Y. (2007): Application of chitosan gel in the treatment of chronic periodontitis. *J Biomed Mater Re*s. Part B. Applied Biomaterials, 80: 290–6.

(7) Cha´vez de Paz, L.E., Resin, A., Howard, K.A., Sutherland, D.S., Wejse, P.L. (2011): Antimicrobial Effect of Chitosan Nanoparticles on Streptococcus mutans Biofilms. *Appl. Environ. Microbiol*, 77 (11): 3892–95.

(8) Kishen, A., Zhilong, S., Shrestha, A., Neoh, K.G. (2008): An Investigation on the Antibacterial and Antibiofilm Efficacy of Cationic Nanoparticulates for Root Canal Disinfection. *J Endod*, 34 (12): 1515–20.

(9) Shingare, P. and Chaugule, V. (2011): Comparative evaluation of antimicrobial activity of miswak, propolis, sodium hypochlorite and saline as root canal irrigants by microbial culturing and quantification in chronically exposed primary teeth. *GERMS*, 1 (1): 12-21.

(10) Hasanin, M.T., Elfeky, S.A., Mohamed, M.B., Amin, R.M. (2018): Production of Well-Dispersed Aqueous Cross-Linked Chitosan-Based Nanomaterials as Alternative Antimicrobial Approach. *J. Inorg. Organomet. Polym. Mater,* 28 (4): 1502–10.

(11) Haapasalo, M., Endal, U., Zandi, H., Coil, J.M. (2005): Eradication of endodontic infection by instrumentation and irrigation solutions. *Endod Topics.*, 10: 77–102.

(12) Krause, T.A., Liewehr, F.R., Hahn, C.L. (2007): The Antimicrobial Effect of MTAD, Sodium Hypochlorite, Doxycycline, and Citric Acid on Enterococcus faecalis. *J Endod*, 33: 28 –30.

(13) Zhang, C., Du, J., Peng, Z. (2015): Correlation between Enterococcus faecalis and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. *J Endod*, 41 (8): 1207-13.

(14) Tabassum, S. and Khan, F.R. (2016): Failure of endodontic treatment: The usual suspects. *Eur J Dent.*, 10 (1): 144–7.

(15) Zou, L., Shen, Y., Li, W., Haapasalo, M. (2010): Penetration of Sodium Hypochlorite into Dentin. *J Endod*, 36: 793–96.

(16) Duke, E.S. (2003): Has dentistry moved into the nanotechnology era? *Compend Contin Educ Dent*., 24: 380– 2.

(17) Lin, K.L., Chang, J., Chen, G.F., Ruan, M.L., Ning, C.Q. (2007): A simple method to synthesize single-crystalline b-wollastonite nanowires. *J Cryst Growth.*, 300: 267–71.

(18) Vahabi, S. and Mardanifar, F. (2014): Applications of nanotechnology in dentistry: a review. *J Dent Sch.*, 32: 228–39.

(19) Roshdy, N.N., Kataia, E.M., Helmy, N.A. (2018): Assessment of Antibacterial Activity of 2.5% NaOCl, Chitosan Nano-Particles against Enterococcus Faecalis Contaminating Root Canals with and without Diode Laser Irradiation: An in Vitro Study. *Acta Odontol. Scand*., 77 (1): 39–43.

(20) Moukarab, D.A.A. (2020): Evaluation of Antimicrobial Activity of Manually Agitate (Nano-Chitosan and Nano- Propolis) against Enterococcus Faecalis in Comparison with Sodium Hypochlorite: An in-Vitro Study. *Egypt. Dent. J*., 66 (1): 587–96.

(21) Podar, R., Kulkarni, G.P., Dadu, S.S., Singh, S., Singh, S.H. (2015): In vivo antimicrobial efficacy of 6% Morinda Citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants. *J Endod*, 9: 529- 34.

(22) Smith, C.S., Setchell, D.J., Harty, F.J. (1993): Factors influencing the success of conventional root canal therapy-a five-year retrospective study. *Int Endo J.*, 26: 321-33.

(23) De Souza, P.F., Goncalves, L.C.O., Marques, A.A.F., Junior, E.C.S., Garcia, L.F.R., de Carvalho, F.M.A. (2015): Root canal retreatment using reciprocating and continuous rotary nickel-titanium instruments. *J Europ Dent.*, 9 (2): 234-39.

(24) Peters, L.B., Wesselink, P.R., Moorer, W.R. (1995): The fate and the role of bacteria left in root dentinal tubules. *Int Endo J*., 28: 95-9.

(25) Siqueira, J.F. and Rôças, I.N. (2019): Microbiology of Apical Periodontitis. *Essential Endodontology*, 91–142.

(26) Banos, J.E., Bosch, F., Canellas, M., Bassols, A., Ortega, F., Bigorra, J. (1989): Acceptability of visual analogue scales in the clinical setting: A comparison with verbal rating scales in postoperative pain. Methods Find *Exp Clin Pharmacol*, 11 (2):123-7.

(27) Williamson, A. and Hoggart, B. (2005): Pain: a review of three commonly used pain rating scales. *J Clin Nurs.*, 14 (7): 798-804.

(28) Viyera, P.J. and Guardado, A. (2009): Incidence and Severity of Post-Operative Pain Following Root Canal Treatment of Teeth with Non-Vital Pulps Using Hand and Rotary Instrumentation Techniques. *Endo*, no. August: 27–30.

(29) Wang, C., Xu, P., Ren, L., Dong, G., Ye, L. (2010): Comparison of Post-Obturation Pain Experience Following One-Visit and Two-Visit Root Canal Treatment on Teeth with Vital Pulps: A Randomized Controlled Trial. . *Int Endo J.*, 43 (8): 692–97.

(30) Kara Tuncer, A. and Gerek, M. (2014): Effect of working length measurement by electronic apex locator or digital radiography on postoperative pain: a randomized clinical trial. *J Endod*, 40: 38–41.

(31) Kandemir Demirci, G. and Calıskan, M.K. (2016): A prospective randomized comparative study of cold lateral condensation versus core/gutta-percha in teeth with periapical lesions. *J Endod*, 42: 206–10.

(32) El-Baz, A.A. and Ahmed, G.M. (2017): Effect of nano-silver irrigating solution on post-operative pain following single visit endodontic therapy: A prospective randomized clinical trial. *Egypt. Dent. J*., 63 (4): 3403-12.

(33) Shrestha, A., Zhilong, S., Gee, N.K., Kishen, A. (2010): Nanoparticulates for Antibiofilm Treatment and Effect of Aging on Its Antibacterial Activity. *J Endod*, 36: 1030–35.

(34) Jaiswal, N., Sinha, D.J., Singh, U.P., Singh, K., Jandial, U.A., Goel, S. (2017): Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on Enterococcus faecalis biofilm: An in vitro study. *J Clin Exp Dent.*, 9 (9): e1066-74.

(35) Hassan, H.Y., Mahmoud, N.F., Zakeer, S. (2017): Evaluation of Antimicrobial Efficacy of Four Tested Solutions Against Enterococcus Faecalis, an in Vitro Study. *Med. J. Cairo Univ.*, 85 (8): 3107–12.

(36) Shrestha, A. and Kishen, A. (2016): Antibacterial Nanoparticles in Endodontics: A Review. *J Endod*, 42 (10): 1417–26.

(37) Simanjuntak, P.A., Djauharie, N., Nursasongko, B. (2019): Antibacterial effectiveness of 2% chitosan and 2% chlorhexidine against enterococcus faecalis in biofilm (laboratory experiment). *Int J App Pharm*, 11 (1): 44-48.

(38) Dychdala, G.R. (1991): Chlorine and chlorine compounds. In: Disinfection, sterilization and prevention, 4th ed., p. 133, Block SS editor, Philadelphia, lea and Febiger.

(39) Darcey, J., Jawad, S., Taylor, C., Roudsari, R.V., Hunter, M. (2016): Modern Endodontic Principles Part 4: Irrigation. Dental Update; 43 (1): 20–33.

(40) Alonso-Ezpeleta, L.O., Gasco-Garcia, C., Castellanos-Cosano, L., Martín- González, J., López-Frías, F.J., Segura-Egea, J.J. (2012): Postoperative pain after one-visit root-canal treatment on teeth with vital pulps: Comparison of three different obturation techniques. *Med Oral Patol Oral Cir Bucal*, 17 (4):e721-7.

(41) Struszcyzk, M.H. (2002): Chitin and chitosan: Part II. Applications of chitosan. *Polimery*, 47: 396-401.

(42) Abbas, A.O. (2010): Chitosan for Biomedical Applications PhD (Doctor of Philosophy) Thesis. USA: University of Iowa.

(43) Hafner, A., Lovrić, J., Romić, M.D., Juretić, M., Pepić, I., Cetina-Čižmek, B., Filipović-Grčić, J. (2015): Evaluation of cationic nanosystems with melatonin using an eye-related bioavailability prediction model. *Eur J Pharm Sci*, 75: 142-150.

(44) Kean, T. and Thanou, M. (2010): Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev.*, 62 (1): 3-11.

(45) Wei, R. , Wang, H., Han, J., Zhao, S., Dumbleton, J., Agarwal, P., Zhang, W., Zhao, G. , Yu, J. , Zynger, D.L., Lu, X. , He, X. (2015): Chitosan-Decorated Doxorubicin-Encapsulated Nanoparticle Targets and Eliminates Tumor Reinitiating Cancer Stem-like Cells . *ACS Nano*, 9 (6): 5725-40.

(46) Caviedes-Bucheli, J., Castellanos, F., Vasquez, N., Ulate, E., Munoz, H.R. (2016): The influence of two reciprocating single-file and two rotary-file systems on the apical extrusion of debris and its biological relationship with symptomatic apical periodontitis. A systematic review and meta-analysis. *Int. Endod. J.*, 49: 255–70.