



Volumetric Assessment of Root Canal Obturation Using 3% Nano-Chitosan versus Zinc Oxide Eugenol (ZOE) and Iodoform-Calcium Hydroxide (Metapex), in Primary Root Canals Shaped with Rotary versus Manual Methods: A Preliminary In-Vitro Spiral CT Study

Elahe Babashahi¹, Maryam Mohmadi Kartalaie^{2*}, Leila Basir¹, Vahid Rakhshan³

1. Department of Pediatric Dentistry, School of Dental Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Pediatric Dentistry, Kashan University of Medical Sciences, Kashan, Iran
3. Dentist, Private Practice, Tehran, Iran

Article Info

Article type:
Original Article

Article History:

Received: 3 June 2018
Accepted: 13 November 2018
Published: 20 January 2019

* Corresponding author:

Department of Pediatric Dentistry,
Kashan University of Medical Sciences,
Kashan, Iran

Email: dr.dentist1980@yahoo.com

ABSTRACT

Objectives: In this study, chitosan was introduced and used as a substitute for pulpctomy obturation against conventional materials: zinc oxide eugenol (ZOE) and iodoform-calcium hydroxide (Ca(OH)₂). Efficacies of rotary versus manual instrumentations were also compared.

Materials and Methods: This preliminary study was performed on 152 intact non-resorbed root canals of primary molars divided into rotary (n=78) versus hand-instrumentation (n=74) and into ZOE (n=53), iodoform-Ca(OH)₂ (n=50), and 3% nano-chitosan (n=49). Canals were cleaned/shaped using hand or rotary files. Canal spaces were measured using spiral computed tomography and obturated using the three materials. The percentages of obturation volume (POV) were estimated. Rotary and manual instrumentations were compared in terms of canal spaces before and after obturation. Three obturation materials were also compared regarding canal spaces after obturation ($\alpha=0.05$).

Results: Average POVs of materials were 96.54% (ZOE), 97.87% (Metapex), and 74.74% (nano-chitosan; $P=0.000$). POV of chitosan differed from the other two ($P<0.001$) but the other two were similar ($P=0.896$). Average POVs were 91.46% (manual) and 88.51% (rotary); the difference was not significant ($P=0.322$). Pre-obturation spaces of canals for different methods were 3.89 mm³ (manual) and 3.26 mm³ (rotary); the difference was significant ($P=0.013$). Two-way ANCOVA showed a significant effect of materials ($P<0.001$) but not root length ($P=0.585$) or shaping methods ($P=0.362$) on POVs.

Conclusion: Nano-chitosan showed a considerable success rate but it still needs reformulation as it was weaker than the extremely successful commercial competitors. Rotary instrumentation can provide results similar to hand-filing in terms of POV although it might yield smaller canals.

Keywords: Chitosan; Primary Teeth; Pulpctomy; Root Canal Obturation

- **Cite this article as:** Babashahi E, Mohmadi Kartalaie M, Basir L, Rakhshan V. Volumetric Assessment of Root Canal Obturation Using 3% Nano-Chitosan versus Zinc Oxide Eugenol (ZOE) and Iodoform-Calcium Hydroxide (Metapex), in Primary Root Canals Shaped with Rotary versus Manual Methods: A Preliminary In-Vitro Spiral CT Study. *Front Dent.* 2019;16(1):45-54. doi: 10.18502/ffd.v16i1.1108

INTRODUCTION

The purpose of root canal therapies in permanent and primary teeth (i.e., pulpectomy) is to eliminate contaminated tissues, canal microorganisms, and their products from the canal through mechanical and chemical cleansing and debridement using hand instruments or nickel-titanium (NiTi) rotary systems [1,2]. Due to the complicated anatomy of root canals (depending on numerous factors such as existence of accessory canals, isthmuses, partial root canal connection, and morphological variations), cleaning and shaping the canals can contribute to half of the bacterial reduction; in primary teeth (molars in particular), these morphological limitations are more severe, their roots are more slender and longer (compared to their crowns), and unlike permanent roots, they undergo physiologic resorption which increases the probability of perforation during root canal therapies; all of these factors make mechanical bacterial reduction difficult [3-5]. Therefore, antimicrobial materials are used to make sure the number of bacteria is considerably reduced in inaccessible parts of root canals [6]. Despite all these efforts, a great deal of microorganisms or debris remains after mechanical and chemical treatments [7]. This calls for additional procedures such as sealing the remaining microorganisms using antimicrobial obturation materials [5]. Ideal pulpectomy obturation materials should be antimicrobial. Since obturation materials can exit the apex and distribute locally and systemically [8], they need to be biocompatible. They also should be resorbable at speeds close to the physiologic root resorption [9]. The most common obturation materials in primary teeth are zinc oxide eugenol (ZOE) and compounds of iodoform and calcium hydroxide ($\text{Ca}(\text{OH})_2$) [5]. ZOE is the most commonly used substance that was recommended in 2008 by the American Academy of Pediatric Dentistry (AAPD) for treatment in primary teeth [10]. The success rate reported for ZOE varies from 65% to 100%, with an average of 83%, and there is no significant difference between the success rates of ZOE and calcium hydroxide or iodoform formulations [9]. The main combinations of iodoform-based pastes are parachlorophenol, camphor, and menthol; they are converted to KRI paste by addition of iodoform. By adding calcium hydroxide, zinc oxide (ZnO), thymol

and lanolin to the KRI, a Maisto paste is obtained. Iodoform compounds are antibacterial, resorbable, and safe to permanent teeth germination [11]. Advantages of KRI paste over ZOE include easier application, faster resorption, and higher antibacterial activities [12] with an overall success rate of about 84% [13]. Calcium hydroxide is another useful pulpectomy root filling material with success rates of about 88% [10]. However, these materials have their own limitations, and there is room for the introduction of other antimicrobial agents with similar or better biocompatibility. For instance, both KRI and ZOE are cytotoxic; the cytotoxicity of KRI remains for 7 days after its setting, while the cytotoxicity of ZOE reaches the baseline after one day [12]. Moreover, over-filling of ZOE during pulpectomy of primary teeth might reduce the success of the treatment from 83% to 58%, while the probability of over-filling of ZOE paste is high because of the thinness of root canal walls in the interradiolar area [14]. The advantage of using iodoform-containing compounds in dentistry is unclear because of complications such as allergy to iodine derivatives, discoloration of substituted teeth [15], and encephalopathy followed by coma [16]. Unless the uncertainties regarding the safety of iodoform as a root canal filler material are resolved, it is advisable to consider the use of other substances.

A novel agent recently suggested for root canal therapy is chitosan which is derived from deacetylation of chitin and has proper antimicrobial, biocompatibility, and anti-inflammatory properties [17,18]. Chitin (a main part of crustaceans) is the second most available biologic base substance in nature, after cellulose, and hence it can serve as an economic and ecological resource [19]. Chitosan, which is a cationic polysaccharide, has outstanding non-toxicity and biocompatibility and therefore is the subject of ever-increasing recent research [20-22]. Since it has a positive charge, it can attach to bacterial or fungal surfaces with a negative charge and destroy them by increasing their permeability [23]. Besides being effective against a broad range of bacteria and fungi, it might also be effective against viruses and tumoral cells, has anti-inflammatory effects and improves immune response and tissue regeneration [24]; moreover, it might facilitate the anti-abrasive

and anti-erosive influences of Sn²⁺ [25-29]. Therefore, recent research has attempted to benefit from its favorable effects by incorporating it into products such as toothpastes, dentifrices, gum, etc.; such studies have proven it to be effective in reducing the discharge of dental mineral elements and bacterial activity and hence decelerating caries formation or enamel decalcification [20, 30-32]. Antimicrobial properties of chitosan have been shown recently [33-35]. However, it is not known if this material can also effectively fill the canal space in order to seal the debris and microbial remnants. Therefore, this preliminary study was conducted to introduce an experimental nano-chitosan formula and to test its canal-filling efficacy determined using the non-invasive yet accurate method of three-dimensional (3D) spiral computed tomography (CT) in comparison with that of ZOE and an 'iodoform-calcium hydroxide' compound as the most common and successful pulpectomy obturation materials. We also compared two methods of cleaning and shaping (rotary versus hand-instrumentation).

MATERIALS AND METHODS

A total of 48 primary first and second molars (D and E) were selected for this study according to the following inclusion criteria: minimum root length of 8 mm, lack of calcification in the root, and absence of perforation in the root. Canals that had resorptions or calcifications (determined later using CT scan) would be excluded. The final sample consisted of 152 canals. The teeth were prepared according to the Occupational Safety and Health Administration (OSHA) guidelines. The teeth were cleaned and placed in a container (with a biohazard label) containing normal saline and were kept at room temperature. Afterwards, they were autoclaved. All study procedures were performed by one operator.

The teeth were randomly divided into two groups of hand-file and rotary preparation (A and B) with similar distributions of maxillary and mandibular teeth in each group. There were 74 and 78 canals in groups A and B, respectively. In group A, there were 18, 21, 18, and 17 canals from maxillary E, mandibular E, maxillary D, and mandibular D, respectively. In group B, there were 17, 26, 18, and 17 canals from maxillary E, mandibular E, maxillary D, and mandibular D, respectively.

Canal preparation:

Access cavities were prepared in each tooth. A K-file #10 (MANI, Korea) was used to determine the exact root length; the working length was considered 1 mm shorter than the apical foramen. In group A (n=74), canals were prepared by K hand-file (MANI) to size 30 by irrigation with saline and 1% sodium hypochlorite (NaOCl) between different file sizes; then, they were dried using sterilized paper cones [36]. In group B (n=78), canals were prepared using rotary files (Mtwo, VDW Co., Munich, Germany) up to size 25 at a 4% convergence with saline and 1% NaOCl irrigation; they were dried using sterile paper cones.

Determining the volume of prepared (empty) root canals using spiral CT scan:

To determine the prepared volume of root canal space, a spiral CT scan was taken from all teeth. First, all teeth were mounted by the root within a red wax block. Then, they were subjected to high-resolution CT scan at a slow speed (VCT, GE System, Optima, USA). Vertical and horizontal slices were sectioned on the entire length and width of the teeth with a thickness of 0.625 mm/slice at a table speed of 0.5 and Kilovoltage peak (kVp) of 140. The scanned data were transferred to GE System Optima software, and the surface areas of canal sections were measured in each slice; the volume of canal space was measured by multiplying the surface area of the cross-section of the canal in each axial slice by 0.625 mm and summing up the volumes of all slices. In order to eliminate measurement error, other sections were assessed, and the floor of the pulp chamber and its direction (in order to determine the vertical axis) were determined.

Obturation:

After excluding canals with CT signs of calcification or resorption, 152 canals remained; these were divided into three subgroups of 1, 2, and 3, each with a balanced distribution of canal types and tooth types. The canals were obturated by a combination of intracanal injection and use of Lentulo spiral (Medin, Korea) attached to a low-speed handpiece, in sizes 20 to 35 based on the size of the root canal. When the obturation material was repelled to the pulp chamber, the canals were assumed as filled; afterwards, a wet cotton

wool was used to gently compress the substance into the root canal. Ultimately, a dressing (Zonalin, Kemdent, Wiltshire, UK) was applied over the filled canal. The used obturation materials in groups 1, 2, and 3 were respectively ZOE (Kemdent, Wiltshire, UK, n=53 canals), Metapex (Meta Biomed Co. Ltd., Cheongju, Korea, n=50 canals), and an experimentally produced 3% nano-chitosan gel (Faculty of Pharmacology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, n=49 canals).

Preparation of experimental 3% nano-chitosan gel:

This material is being introduced as a non-commercialized experimental material. First, 0.1% chitosan solution was prepared: 10 g of 1% acetic acid solution (BDH Laboratory Supplies, Poole Dorset, UK) was mixed with 10 mg of chitosan powder with a viscosity of less than 25 cP (ChitoClear, Primex Biochemicals; Haugesund, Norway). The resulting compound was kept for 24 hours in a container in order to completely hydrate the chitosan.

Afterwards, 0.1% Sodium Tripolyphosphate (TPP) solution was prepared by mixing TPP powder (Acros Organics, USA) with distilled water.

The third stage was to prepare chitosan nanoparticles by mixing chitosan solution within a stirrer blending at 1400 rpm (revolutions per minute) with TPP solution dripped until an opaque suspension was created. Afterwards, the suspension was made radiopaque by adding 10% barium sulfate (Merck, Germany) to the suspension. Finally, a paste-like 3% nano-chitosan gel was created by gradually adding 0.45 g of chitosan powder (ChitoClear) with 500 cP to the suspension and waiting for 24 hours for the gel to be completely hydrated [24]. The produced material was immediately used.

Estimation of the percentage of obturated canal space:

After obturation, teeth were subjected to CT scan, exactly as mentioned above. The cross-section of obturated material in each axial scan was computed, and the volume of obturated material in that slice was estimated. The volumes of all slices were summed up for each canal. The percentage of obturation volume (POV) was calculated for each canal as filled space \times 100 / total canal space.

Statistical analysis:

Descriptive statistics and 95% confidence intervals (CI) were calculated. Data normality was confirmed using Kolmogorov-Smirnov test. Groups were compared using one-way analysis of variance (ANOVA), independent-samples t-test, and Tukey test in terms of pre-obturation volumes and POVs among different tooth types, different root types, different materials, and different methods. Effects of different obturation materials and methods together with root lengths on POV were assessed using two-way analysis of covariance (ANCOVA) of SPSS 25 (IBM Corp., Armonk, NY, USA). P values \leq 0.05 were considered significant.

RESULTS

Mean \pm standard deviation (SD) of pre-filling canal space volume was 3.57 ± 1.59 mm³ (95% CI = 3.31 to 3.82) in all 152 roots. Mean \pm SD of POV was $89.95 \pm 18.31\%$ (95% CI = 3.31 to 3.82) in all roots. ANOVA did not show any significant difference among various tooth types (first and second primary molars in the maxilla or the mandible) in terms of pre-obturation canal space volume (P=0.115) or POVs (P=0.378; Table 1). ANOVA did not detect any significant difference among different root types (mesiobuccal1, mesiolingual, distobuccal, distolingual, palatal, distal, and mesiobuccal2) in terms of POVs (P=0.879; Table 2); however, it detected a significant difference among pre-obturation canal space volumes of different root types (P=0.000; Table 2).

Although pre-obturation canal spaces did not differ among three material groups (P=0.255; Table 3), these materials showed significantly different POVs after obturation (P=0.000; Table 3). Chitosan had a significantly smaller mean POV compared to ZOE or Metapex (Tukey, P=0.000) but there was no significant difference between ZOE and Metapex (P=0.896). Independent-samples t-test did not show any significant difference between POVs of the shaping methods (P=0.322; Table 4). However, it showed that hand-filing can create a more spacious pre-obturation canal compared to the rotary system (P=0.013; Table 4). Two-way ANCOVA showed a significant effect of materials (P=0.000) but not root length (P=0.585) or cleaning methods (P=0.362) on POVs. The interaction of filling materials and cleaning methods was non-significant (P=0.181).

Table 1. Descriptive statistics for pre-obturation canal space volume (mm³) and POVs (%) among various teeth

| | Tooth | N | Mean | SD | 95% CI | Minimum | Maximum |
|---------------------------|--------------|----|-------|-------|-------------|---------|---------|
| Pre-Filling Volume | Maxillary E | 35 | 3.48 | 1.57 | 2.94-4.01 | 0.76 | 8.45 |
| | Maxillary D | 36 | 3.54 | 1.47 | 3.05-4.04 | 0.76 | 6.56 |
| | Mandibular E | 47 | 3.26 | 1.46 | 2.83-3.69 | 0.29 | 6.74 |
| | Mandibular D | 34 | 4.12 | 1.83 | 3.48-4.76 | 1.54 | 7.98 |
| POV | Maxillary E | 35 | 88.72 | 20.83 | 81.56-95.87 | 24.18 | 100 |
| | Maxillary D | 36 | 92.76 | 11.65 | 88.82-96.70 | 62.37 | 100 |
| | Mandibular E | 47 | 91.66 | 18.62 | 86.20-97.13 | 3.69 | 100 |
| | Mandibular D | 34 | 85.87 | 20.72 | 78.64-93.10 | 34.26 | 100 |

POV: Percent of Obturated Volume, SD: Standard Deviation, CI: Confidence Interval

Table 2. Descriptive statistics for pre-obturation canal space volume (mm³) and POVs (%) among various roots

| | Canal | N | Mean | SD | 95% CI | Minimum | Maximum |
|---------------------------|--------------|----|-------|-------|-------------|---------|---------|
| Pre-Filling Volume | Mesiobuccal | 50 | 3.74 | 1.55 | 3.30-4.18 | 1.63 | 7.98 |
| | Mesiolingual | 21 | 2.90 | 1.28 | 2.32-3.49 | 0.29 | 5.72 |
| | Distobuccal | 41 | 3.08 | 1.42 | 2.63-3.52 | 0.76 | 6.53 |
| | Distolingual | 15 | 3.39 | 1.42 | 2.61-4.17 | 1.89 | 6.36 |
| | Palatal | 22 | 4.62 | 1.73 | 3.85-5.39 | 1.53 | 8.45 |
| | Distal | 2 | 6.32 | 1.21 | -4.52-17.16 | 5.47 | 7.18 |
| | Mesiobuccal2 | 1 | 3.23 | --- | --- | 3.23 | 3.23 |
| POV | Mesiobuccal | 50 | 87.91 | 21.52 | 81.79-94.02 | 3.69 | 100 |
| | Mesiolingual | 21 | 92.83 | 16.75 | 85.21-100 | 42.13 | 100 |
| | Distobuccal | 41 | 92.24 | 14.44 | 87.68-96.79 | 49.57 | 100 |
| | Distolingual | 15 | 89.03 | 20.43 | 77.71-100 | 34.26 | 100 |
| | Palatal | 22 | 88.94 | 18.37 | 80.80-97.09 | 24.18 | 100 |
| | Distal | 2 | 88.63 | 16.08 | -55.86-100 | 77.26 | 100 |
| | Mesiobuccal2 | 1 | 76.36 | --- | --- | 76.36 | 76.36 |

POV: Percent of Obturated Volume, SD: Standard Deviation, CI: Confidence Interval

Table 3. Descriptive statistics for pre-obturation canal space volume (mm³) and POVs (%) among different materials

| | Agent | N | Mean | SD | 95% CI | Minimum | Maximum |
|---------------------------|----------|----|-------|-------|-------------|---------|---------|
| Pre-Filling Volume | ZOE | 53 | 3.65 | 1.65 | 3.19-4.10 | 0.29 | 8.45 |
| | Metapex | 50 | 3.27 | 1.58 | 2.82-3.72 | 0.76 | 6.74 |
| | Chitosan | 49 | 3.78 | 1.54 | 3.34-4.22 | 1.63 | 7.98 |
| POV | ZOE | 53 | 96.54 | 9.70 | 93.87-99.21 | 46.22 | 100 |
| | Metapex | 50 | 97.87 | 8.20 | 95.54-100 | 47.34 | 100 |
| | Chitosan | 49 | 74.74 | 23.14 | 68.10-81.39 | 3.69 | 100 |

POV: Percent of Obturated Volume, SD: Standard Deviation, CI: Confidence Interval, ZOE: Zinc Oxide Eugenol

Table 4. Descriptive statistics for pre-obturation canal space volume (mm³) and POVs (%) between hand-instrumentation and rotary system

| | Method | N | Mean | SD | 95% CI | Minimum | Maximum |
|---------------------------|--------|----|-------|-------|-------------|---------|---------|
| Pre-Filling Volume | Manual | 74 | 3.89 | 1.57 | 3.53-4.26 | 0.29 | 7.98 |
| | Rotary | 78 | 3.26 | 1.57 | 2.90-3.61 | 0.76 | 8.45 |
| POV | Manual | 74 | 91.46 | 16.34 | 87.68-95.25 | 34.26 | 100 |
| | Rotary | 78 | 88.51 | 20.01 | 84.00-93.02 | 3.69 | 100 |

POV: Percent of Obturated Volume, SD: Standard Deviation, CI: Confidence Interval

Table 5. Descriptive statistics for POVs (%) among three materials in each of the two subgroups: hand-instrumentation and rotary system

| Method | Material | N | Mean | SD | SE | 95% CI | Minimum | Maximum |
|--------|----------|----|-------|-------|------|-------------|---------|---------|
| Manual | ZOE | 28 | 95.58 | 12.61 | 2.38 | 90.69-100 | 46.22 | 100 |
| | Metapex | 24 | 97.81 | 10.75 | 2.19 | 93.27-100 | 47.34 | 100 |
| | Chitosan | 22 | 79.31 | 19.34 | 4.12 | 70.73-87.89 | 34.26 | 100 |
| Rotary | ZOE | 25 | 97.62 | 4.76 | 0.95 | 95.65-99.58 | 84.71 | 100 |
| | Metapex | 26 | 97.93 | 5.05 | 0.99 | 95.89-99.97 | 82.28 | 100 |
| | Chitosan | 27 | 71.02 | 25.57 | 4.92 | 60.90-81.13 | 3.69 | 100 |

POV: Percent of Obturated Volume, ZOE: Zinc Oxide Eugenol, SD: Standard Deviation, SE: Standard Error, CI: Confidence Interval

One-way ANOVA and Tukey post hoc tests were used to compare the POVs of different materials in each of the subgroups pertaining to two methods of shaping (rotary and manual).

There were differences among the three materials in the rotary subgroup ($P=0.000$) and in the manual subgroup ($P=0.000$; Table 5). In the manual filing subgroup, Tukey test detected significant differences between chitosan with either of the other two materials ($P\leq 0.001$) but not between ZOE and Metapex ($P=0.845$). Similarly, in the rotary subgroup, there were significant differences between chitosan with either of the other two materials ($P=0.000$) but not between ZOE and Metapex ($P=0.997$; Table 5).

DISCUSSION

Root canal therapy in the primary dentition is different from that in the permanent dentition from some aspects. The first difference is related to the morphology of the primary dentition, which is different from that of permanent teeth and has more lateral canals and extra canals, and hence is more difficult to clean. The second problem in primary teeth is the flow of the filling substance in primary canals which are narrower and more twisted than permanent canals.

primary dentition, which might increase the probability of over-filling, which calls for higher biocompatibility of root canal fillers in primary teeth [37]. Root filling materials should also be bacteriostatic; compounds of iodoform and calcium hydroxide are slightly antibacterial [4]. In some cases, bactericidal agents are added to their formulations in order to improve the treatment success. The usual primary root filling materials (such as ZOE and calcium hydroxide compounds) provide proper antibacterial properties and biocompatibility [37].

Primary root obturation materials should also be resorbable and should not induce inflammation in the permanent dental bud [9]. However, they should also be capable of flowing through narrow parts of the canal, adhering well to the dentin on canal walls, and hence producing the minimum void [38]. The findings of this study suggested that ZOE and Metapex were highly effective in terms of the volume of the filled canal, and a high sealing potential can contribute to proper clinical success as well [39-41]. In the present study, we introduced and assessed, for the first time, the flow of chitosan as a new bioactive and highly biocompatible root-filling material with antibacterial properties in comparison to the routine primary root filling materials that were shown to be successful previously and were economic and convenient [36]. This particular formulation of nano-chitosan (being tested for the first time) was successful as well although not as perfectly polished as its commercial counterparts. The comparison of pre- and post-obturation CT scans showed that all three materials filled the canal space considerably (though not perfectly); these results were similar with previous studies [36,42]. Void occurrence is inevitable although using pressure syringes might reduce it [43]. We used the same hybrid method for all three materials, and therefore, eliminated the confounding effect of the filling methods. Metapex and ZOE filled over 95% of the canal space. The insignificant superiority of Metapex over ZOE was consistent in both hand-filed canals and those shaped using rotary instruments. Our results pertaining to ZOE and Metapex were in line with previous findings [36]. However, the extent of root filling was only about 75% with nano-chitosan. The relative lower percentage of nano-chitosan can be due to the preliminary nature of this study as there was no previous study on nano-chitosan in

order for us to be able to optimize its viscosity and wall-adhesion properties based on earlier findings. Still, this experimental formula filled up to about an average of 75% of root canal space, which can be considered a success for a pilot study. Chitosan has an integrated gel structure, which might reduce its adhesion to dentin walls or its flow through narrower canal spaces. Future studies can add various materials to chitosan and/or change its percentage in order to improve its filling potential. For instance, chitosan is acidic and therefore adheres well to metal ions such as calcium [44]; this might allow the production of improved formulations that can provide both better antibacterial properties and improved dentin bonding characteristics [44,45]. The most common obturation material for primary teeth is ZOE paste. Several human and animal studies have reported success rates of about 65-95% in treatment with ZOE. Iodoform-based pastes (such as Metapex and Vitapex which are a combination of iodoform and calcium hydroxide) are recommended by many researchers and can have success rates of about 70-90% [13]. Both of these materials have resorption rates similar to or faster than that of the roots of primary teeth [10].

Techniques of canal preparation and their advantages are a subject of ongoing debate [46]. The session should be brief in pediatric dentistry, and therefore, rotary instruments might be of assistance in this regard if they prove effective clinically.

In this study, hand-filing was compared with rotary instrumentation. Both methods were similar in terms of POVs after obturation. In terms of the canal spaces before obturation, the rotary system led to slightly smaller canals. This was in contrast to previous research reporting similar extents of canal cleaning by these methods [47-49] and might be due to the fact that in this study, the alloys of the rotary and manual files were not similar. Such findings together with other advantages of rotary systems such as following the natural curvature of the canal, mark rotary canal preparation as an efficient yet fast technique which can be recommended for root canal therapy in children who would not tolerate extended sessions [50,51]. Therefore, many clinicians prefer to use rotary systems, especially in canals inaccessible to hand instruments [4].

This pilot study was limited by some factors. A

larger sample size could improve the reliability of the findings; given the high number of partially resorbed primary roots, collecting a greater number of teeth was very difficult. Yet, the current sample size had many canals per each subgroup, and all subgroups were balanced in terms of the tooth and canal types. Additionally, the results of in vitro studies cannot be generalized to clinical conditions, and clinical studies are needed to verify in vitro results once the optimum concentration and formulation of nano-chitosan have been determined in vitro. Another limitation was lack of any previous knowledge about optimum conditions of storage and application of the experimental formula; potential physico-chemical alterations during the storage were not known, and the best method of its application within canals was not determined or standardized yet. Therefore, we immediately used the produced material, without any storage, in order to eliminate the confounding effects associated with its storage. Also, we used both methods of application together to reduce or eliminate the confounding effects. It was better to prepare various percentages of nano-chitosan in order to test which one can provide the highest POVs. Also, it was possible to add different materials to chitosan to improve its physical properties. Moreover, the antibacterial properties of chitosan should have been tested, and the optimum percentage of chitosan that could deliver proper antibacterial and root filling properties at the same time should have been found. However, these were quite beyond the budget of this study and will be addressed in future research. Another limitation was the use of spiral CT scan, while it is recommended to use micro-CT for determination of root canal morphology and assessment of obturation quality [52,53]. However, micro-CT was not available, and the dispersion of spiral CT results confirmed the proper accuracy of spiral CT. Conventional methods of assessment of canal obturation percentage are limited by constraints such as two-dimensional (2D) radiographs being an inaccurate projection of 3D canal space, or disruption and losing a part of the obturation substance during physical sectioning, creating new voids and contributing to false positive errors; moreover, once sectioned, the tooth cannot be used again, and therefore, it is not possible to calculate the canal space both before and after obturation [36].

Therefore, 3D radiographic techniques such as spiral CT can eliminate both of these limitations and also allow spotting the accurate place of voids [36,54,55], which can be used in retreatment of failed root canal therapies [54].

CONCLUSION

The experimental 3% nano-chitosan could fill only about 3/4 of canal space. The commercial ZOE and iodoform-calcium hydroxide materials were on the other hand extremely successful. Their results were comparable to each other either in canals prepared using manual instrumentation or shaped with the rotary system. Compared to the rotary system, the manual instrumentation technique might result in slightly more spacious canals.

REFERENCES

1. Moskovitz M, Sammara E, Holan G. Success rate of root canal treatment in primary molars. *J Dent*. 2005 Jan;33(1):41-7.
2. Crespo S, Cortes O, Garcia C, Perez L. Comparison between rotary and manual instrumentation in primary teeth. *J Clin Pediatr Dent*. 2008 Summer;32(4):295-8.
3. Thomas AM, Chandra S, Chandra S, Pandey RK. Elimination of infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. *J Endod*. 1994 May;20(5):233-5.
4. Dean JA. Treatment of Deep Caries, Vital Pulp Exposure, and Pulpless Teeth, in Dean JA (editor). *McDonald and Avery's Dentistry for the Child and Adolescent*. Maryland Heights, Missouri, USA, Elsevier, 2015:221-53.
5. Bonow MLM, Guedes-Pinto AC, Bammann LL. Antimicrobial activity of drugs used in pulp therapy of deciduous teeth. *Braz Endod J*. 1996;1(1):44-8.
6. Leonardo MR, Silveira FF, Silva LA, Tanomaru Filho M, Utrilla LS. Calcium hydroxide root canal dressing. Histopathological evaluation of periapical repair at different time periods. *Braz Dent J*. 2002;13(1):17-22.
7. Queiroz AM, Nelson-Filho P, Silva LA, Assed S, Silva RA, Ito IY. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex and EndoREZ. *Braz Dent J*. 2009;20(4):290-6.
8. Bartelstone HJ. Radioiodine penetration through intact enamel with uptake by bloodstream and thyroid gland. *J Dent Res*. 1951 Oct;30(5):728-33.
9. Fuks AB, Kupietsky A, Guelmann M. Pulp therapy for the primary dentition, in Casamassimo P, Fields H, McTigue D, Nowak A (editors). *Pediatric Dentistry: Infancy through Adolescence*. Maryland Heights, Missouri, USA, Elsevier, 2013:333-51.
10. Nurko C, Garcia-Godoy F. Evaluation of a calcium hydroxide/iodoform paste (Vitapex) in root canal therapy for primary teeth. *J Clin Pediatr Dent*. 1999 Summer;23(4):289-94.
11. Gould JM. Root canal therapy for infected primary molar teeth-preliminary report. *ASDC J Dent Child*. 1972;39(4):269-73.
12. Wright KJ, Barbosa SV, Araki K, Spangberg LS. In vitro antimicrobial and cytotoxic effects of Kri 1 paste and zinc oxide-eugenol used in primary tooth pulpectomies. *Pediatr Dent*. 1994 Mar-Apr;16(2):102-6.
13. Holan G, Fuks AB. A comparison of pulpectomies using ZOE and KRI paste in primary molars: a retrospective study. *Pediatr Dent*. 1993 Nov-Dec;15(6):403-7.
14. Fuks A, Eidelman E, Pauker N. Root fillings with Endoflas in primary teeth: a retrospective study. *J Clin Pediatr Dent*. 2002 Fall;27(1):41-5.
15. Baumgartner JC, Rosenberg PA, Hoen MM, Lin LM. Treatment of endodontic infections, cysts, and flare-ups, in Baumgartner JC (editor). *Ingle's Endodontics*. Ontario, Canada: B.C. Decker Inc., 2009:690-713.
16. Roy PM, Harry P, Cailleux A, Allain P. Dangers of bismuth iodoform paraffin paste. *Lancet*. 1994 Dec;344(8938):1708.
17. Tharanathan RN, Kittur FS. Chitin--the undisputed biomolecule of great potential. *Crit Rev Food Sci Nutr*. 2003;43(1):61-87.
18. Ikeda T, Yanagiguchi K, Vilorio IL, Hayashi Y. Relationship between lysozyme activity and clinical symptoms following the application of chitin/chitosan in endodontic treatment, in Muzzarelli RAA (editor). *Chitosan Per Os: from Dietary Supplement to Drug Carrier*. Crottammare, Italy, Atec Edizioni, 2000:275-92.
19. Peter MG. Applications and Environmental Aspects of Chitin and Chitosan. *J Macromol Sci A*. 1995;32(4):629-40.
20. Targino AG, Flores MA, dos Santos Junior VE, de Godoy Bené Bezerra F, de Luna Freire H, Galembeck A, et al. An innovative approach to treating dental decay in children. A

- new anti-caries agent. *J Mater Sci Mater Med*. 2014 Aug;25(8):2041-7.
21. Muzzarelli RAA. Chitins and Chitosans as Immunoadjuvants and Non-Allergenic Drug Carriers. *Mar Drugs*. 2010 Feb;8(2):292-312.
 22. Dutta PK, Dutta J, Tripathi VS. Chitin and chitosan: chemistry, properties and applications. *J Sci Ind Res*. 2004;63(1):20-31.
 23. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*. 2003 Nov-Dec;4(6):1457-65.
 24. Sun L, Du Y, Fan L, Chen X, Yang J. Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. *Polymer*. 2006 Mar;47(6):1796-804.
 25. Song XL, Sun YY, Liu Y, Chen Q, Bi XX, Yu JT. [An in vitro evaluation of the effect of carboxymethyl chitosan and its composites against *Enterococcus faecalis* in the root canal]. [Article in Chinese]. *Shanghai Kou Qiang Yi Xue*. 2013 Jun;22(3):265-9.
 26. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, et al. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. *Biomaterials*. 1999 Aug;20(15):1407-14.
 27. Matsunaga T, Yanagiguchi K, Yamada S, Ohara N, Ikeda T, Hayashi Y. Chitosan monomer promotes tissue regeneration on dental pulp wounds. *J Biomed Mater Res A*. 2006 Mar 15;76(4):711-20.
 28. Schlueter N, Klimek J, Ganss C. Effect of a chitosan additive to a Sn 2+-containing toothpaste on its anti-erosive/anti-abrasive efficacy--a controlled randomised in situ trial. *Clin Oral Investig*. 2014 Jan;18(1):107-15.
 29. Costa EM, Silva S, Pina C, Tavoria FK, Pintado MM. Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens. *Anaerobe*. 2012 Jun;18(3):305-9.
 30. Uysal T, Akkurt MD, Amasyali M, Ozcan S, Yagci A, Basak F, et al. Does a chitosan-containing dentifrice prevent demineralization around orthodontic brackets? *Angle Orthod*. 2011 Mar;81(2):319-25.
 31. Hayashi Y, Ohara N, Ganno T, Ishizaki H, Yanagiguchi K. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. *J Dent*. 2007 Nov;35(11):871-4.
 32. Arnaud TM, de Barros Neto B, Diniz FB. Chitosan effect on dental enamel remineralization: an in vitro evaluation. *J Dent*. 2010 Nov;38(11):848-52.
 33. Moghadas L, Shahmoradi M, Narimani T. Antimicrobial activity of a new nanobased endodontic irrigation solution: In vitro study. *Dent Hypotheses*. 2012;3(4):142-6.
 34. Suzuki S, Masuda Y, Morisaki H, Yamada Y, Kuwata H, Miyazaki T. The Study of Chitosan-Citrate Solution as a Root Canal Irrigant: A Preliminary Report. *J Oral Hyg Health*. 2014;2:142.
 35. Imani Z, Imani Z, Basir L, Shayeste M, Abbasi Montazeri E, Rakhshan V. Antibacterial Effects of Chitosan, Formocresol and CMCP as Pulpotomy Medicament on *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus mutans*. *Iran Endod J*. 2018 Summer;13(3):342-50.
 36. Asokan S, Sooriaprakas C, Raghu V, Bairavi R. Volumetric analysis of root canal fillings in primary teeth using spiral computed tomography: an in vitro study. *J Dent Child (Chic)*. 2012 May-Aug;79(2):46-8.
 37. Huang TH, Ding SJ, Kao CT. Biocompatibility of various formula root filling materials for primary teeth. *J Biomed Mater Res B Appl Biomater*. 2007 Feb;80(2):486-90.
 38. Keyes PH, Jordan HV. Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. *Arch Oral Biol*. 1964 Jul-Aug;9:377-400.
 39. Swanson K, Madison S. An evaluation of coronal microleakage in endodontically treated teeth. Part I. Time periods. *J Endod*. 1987 Feb;13(2):56-9.
 40. Madison S, Swanson K, Chiles SA. An evaluation of coronal microleakage in endodontically treated teeth. Part II. Sealer types. *J Endod*. 1987 Mar;13(3):109-12.
 41. Gillen BM, Looney SW, Gu LS, Loushine BA, Weller RN, Loushine RJ, et al. Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: a systematic review and meta-analysis. *J Endod*. 2011 Jul;37(7):895-902.
 42. Anbu R, Nandini S, Velmurugan N. Volumetric analysis of root fillings using spiral computed tomography: an in vitro study. *Int Endod J*. 2010 Jan;43(1):64-8.
 43. Dandashi MB, Nazif MM, Zullo T, Elliott MA, Schneider LG, Czonstkowsky M. An in vitro

- comparison of three endodontic techniques for primary incisors. *Pediatr Dent*. 1993 Jul-Aug;15(4):254-6.
44. Silva PV, Guedes DF, Pécora JD, da Cruz-Filho AM. Time-dependent effects of chitosan on dentin structures. *Braz Dent J*. 2012;23(4):357-61.
45. Busscher HJ, Engels E, Dijkstra RJ, van der Mei HC. Influence of a chitosan on oral bacterial adhesion and growth in vitro. *Eur J Oral Sci*. 2008 Oct;116(5):493-5.
46. Kim HC, Kim HJ, Lee CJ, Kim BM, Park JK, Versluis A. Mechanical response of nickel-titanium instruments with different cross-sectional designs during shaping of simulated curved canals. *Int Endod J*. 2009 Jul;42(7):593-602.
47. Schafer E, Zapke K. A comparative scanning electron microscopic investigation of the efficacy of manual and automated instrumentation of root canals. *J Endod*. 2000 Nov;26(11):660-4.
48. Silva LA, Leonardo MR, Nelson-Filho P, Tanomaru JM. Comparison of rotary and manual instrumentation techniques on cleaning capacity and instrumentation time in deciduous molars. *J Dent Child (Chic)*. 2004 Jan-Apr;71(1):45-7.
49. Azar MR, Mokhtare M. Rotary Mtwo system versus manual K-file instruments: efficacy in preparing primary and permanent molar root canals. *Indian J Dent Res*. 2011 Mar-Apr;22(2):363.
50. Kleier DJ, Averbach R. Comparison of clinical outcomes using a nickel titanium rotary or stainless steel hand file instrumentation technique. *Compend Contin Educ Dent*. 2006 Feb;27(2):87-91; quiz 92, 112.
51. Makarem A, Ravandeh N, Ebrahimi M. Radiographic assessment and chair time of rotary instruments in the pulpectomy of primary second molar teeth: a randomized controlled clinical trial. *J Dent Res Dent Clin Dent Prospects*. 2014 Spring;8(2):84-9.
52. Peters OA, Peters CI, Schonenberger K, Barbakow F. ProTaper rotary root canal preparation: effects of canal anatomy on final shape analysed by micro CT. *Int Endod J*. 2003 Feb;36(2):86-92.
53. Hammad M, Qualtrough A, Silikas N. Evaluation of root canal obturation: a three-dimensional in vitro study. *J Endod*. 2009 Apr;35(4):541-4.
54. Barletta FB, de Sousa Reis M, Wagner M, Borges JC, Dall'Agnol C. Computed tomography assessment of three techniques for removal of filling material. *Aust Endod J*. 2008;34(3):101-5.
55. Reuben J, Velmurugan N, Kandaswamy D. The evaluation of root canal morphology of the mandibular first molar in an Indian population using spiral computed tomography scan: an in vitro study. *J Endod*. 2008 Feb;34(2):212-5.