

Keywords

Chlorine dioxide
Irrigation
Maleic acid
Microhardness
Surface roughness

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Received: 06.02.15

Accepted: 06.08.15

doi: 10.1922/EJPRD_1445Ballal06

Evaluation of Chlorine Dioxide Irrigation Solution on the Microhardness and Surface Roughness of Root Canal Dentin

ABSTRACT

The aim of this study was to evaluate the effect of chlorine dioxide and various other more common irrigation solutions on the microhardness and surface roughness of root canal dentin. Fifty human maxillary central incisors were sectioned longitudinally and treated for 1 minute with 5 ml of the following aqueous solutions (v/v%): Group 1: 13.8% chlorine dioxide, Group 2: 17% ethylene diamine tetraacetic acid (EDTA). Group 3: 7% maleic acid, Group 4: 2.5% sodium hypochlorite (5ml/min), Group 5: Saline (control). Specimens were subjected to microhardness and surface roughness testing. Chlorine dioxide and sodium hypochlorite reduced the microhardness more than other test agents. The highest surface roughness was produced with maleic acid. Chlorine dioxide should be used cautiously during chemomechanical preparation of the root canal system in order to prevent untoward damage to the teeth.

INTRODUCTION

The success of root canal therapy depends on the quality of instrumentation, irrigation, disinfection and three-dimensional obturation of the root canal system. Mechanical instrumentation of the root canal produces a smear layer that covers the dentinal tubules.¹ Meta-analysis of leakage studies concluded that the removal of the smear layer improves the hermetic seal of the root canal system.² The smear layer can be removed using various chelating agents like ethylene diamine tetraacetic acid (EDTA), citric acid and MTAD [mixture of tetracycline (3% doxycycline hyclate), acid (4.25% citric acid) and a detergent (Tween 80)].^{3,4,5} The combination of EDTA and sodium hypochlorite (NaOCl) is most commonly used for smear layer removal.⁶ However, 7% maleic acid (MA) in combination with 2.5% sodium hypochlorite has been found to be significantly better than 17% EDTA in removal of the smear layer, especially from the apical third of the root canal system.⁷ It is also known to be less cytotoxic compared to 17% EDTA.⁸ Peters *et al.* reported that, mechanical instrumentation of the root canal system leaves 35-53% of areas unaffected.⁹ Lack of instrumentation may harbor pulpal remnants, biofilm structure, and their by-products,^{10,11} which might prevent the adaptation of the obturation material and result in periradicular infection.^{12,13,14} Hence, irrigation with efficient antibacterial agent is an essential part of root canal therapy, since it allows for better disinfection than achieved solely by mechanical instrumentation.¹⁵

Chlorine dioxide (ClO_2) a new endodontic irrigant is chemically similar to chlorine or hypochlorite. ClO_2 makes a likely substitute for the hypochlorite due to its reduced toxicity and irritating effect when applied to the human body. ClO_2 is currently used in food processing, surface disinfection, water treatment, veterinary care, dental waterline treatment, and some mouth rinses.¹⁶⁻²⁰ According to the manufacturer, it is known to have tuberculocidal, bactericidal, virucidal, and fungicidal properties and due to its powerful oxidizing properties, it is known to kill bacteria by disrupting the transport of nutrients across the cell wall (21). ClO_2 has recently come under consideration as a possible root canal irrigant because of its reported antibacterial activity and biocompatibility.^{22,23,24} It was reported that, ClO_2 and NaOCl were both effective in eliminating *E. faecalis* from the dentinal disks within 30 min.²² Cobankara *et al.*²⁵ demonstrated that 13.8% ClO_2 was as effective as 5.25% NaOCl in dissolving bovine pulp tissue. The irrigating solutions, when used in root canal dentin, can cause changes in the microstructure of the dentin and changes in the Ca:P ratio. Microhardness, surface roughness, permeability and solubility of the root canal dentin are affected by changes in the Ca:P ratio, which in turn inhibits the resistance of root canal dentin to bacterial ingress, thus permitting coronal leakage.²⁶ The sealing ability and adhesion of resin-based root canal sealers depends on the mineral content of the root canal dentin. Hence, any changes in the mineral content may adversely affect the properties of resin-based materials.²⁶ Studies have shown that, EDTA, MA and NaOCl are capable of decreasing the microhardness of root canal dentin.^{27,28} So far there are no studies evaluating the effect of ClO_2 on the microhardness and surface roughness of root canal dentin. Hence, the purpose of this *in vitro* study was to evaluate the effect of 13.8% ClO_2 , 7% MA, 17% EDTA and 2.5% NaOCl solutions on the microhardness and the surface roughness of human root canal dentin.

MATERIALS AND METHODS

Fifty extracted human maxillary central incisors were selected. Selection of teeth was based on their relative dimensions and similarity in morphology. Ethical clearance was obtained from the institutional review board of Manipal University. Superficial soft tissues were removed with a brush and the teeth were stored in 0.2% sodium azide at 4°C. The teeth with caries, cracks and dilacerations were excluded. Teeth were decoronated at the cemento-enamel junction using a high-speed diamond point under water cooling, after which the pulp tissue was removed using barbed broach. Each root was then sectioned longitudinally with a low speed diamond disc, separating each root into buccal and palatal halves. The root segments were then horizontally embedded in auto polymerizing acrylic resin, leaving their dentin exposed to facilitate ma-

nipulation and improve metallographic preparation. The root canal dentin surfaces of the specimens were ground with a series of silicon carbide abrasive papers (500, 800, 1000, 1200 grit) under distilled water and finally polished with 0.1 μm alumina suspension on a rotary felt disk. 100 specimens were then divided into five groups (n=20) and were treated for 1 minute with 5 ml of the following aqueous solutions (v/v%): Group 1: 13.8% chlorine dioxide, Group 2: 17% ethylene diamine tetraacetic acid (EDTA). Group 3: 7% maleic acid, Group 4: 2.5% sodium hypochlorite, and Group 5: 0.9 % saline (control).

All the specimens in each group were immersed in the respective irrigating solutions and then washed thoroughly with distilled water. Every group was divided into two sub groups of 10 specimens each. In group 1a, 2a, 3a, 4a and 5a specimens were used to determine the microhardness of the root canal dentin using Knoop hardness tester. The indentations were made with a Knoop diamond indenter at three different locations. The locations were chosen at 0.5 mm level to the root canal wall in the coronal, middle and apical third of the roots. The indentations were made on each specimen using 500 g load and 40 s dwell time. The diamond shaped indentations were captured with an optical microscope linked to a digital camera and image analysis software, allowing the accurate digital measurement of the indent. The average length of the two diagonals was used to calculate the microhardness value. The representative hardness value for each specimen was obtained as the average of the results of the three indentations.

In groups 1b, 2b, 3b, 4b and 5b specimens were used for the determination of surface roughness (R_a , μm) of the root canal dentin using Atomic force microscopy (AFM) (Veeco, Santa Barbara, CA). The AFM probe was operated in contact mode to image the root canal dentin surfaces using silicon nitrate pyramidal tips. The instrument was calibrated before the measurements using polyethylene spheres of known diameter. AFM images were analyzed using an image processing software to calculate the surface roughness. All AFM scans were performed by a single trained operator, who was blind towards the test groups. The tip was moved across the root canal dentin surface at three different locations (10 × 10 μm^2) on each specimen, and the roughness was measured. The values were averaged to produce a single R_a value for each dentin specimen.

STATISTICAL ANALYSIS

The group comparison of the microhardness and surface roughness values were statistically analyzed using Kruskal Wallis test and intergroup comparison was done using Mann Whitney U test.

RESULTS

Microhardness

The mean microhardness at the coronal, middle and apical thirds of the root canal system following the different irrigation regimens is shown in Figure 1. The control group (saline) had a statistically significant lesser effect on the microhardness compared to other test agents in the coronal, middle and apical third ($p < 0.001$). A comparison between 13.8% ClO_2 and 17% EDTA showed that, there was a statistically significant difference in the coronal ($p < 0.001$), middle ($p = 0.003$) and apical third ($p < 0.001$) thirds, in which 13.8% ClO_2 reduced the microhardness more compared with 17% EDTA. When 13.8% ClO_2 was compared with 7% MA, there was no significant difference between the two groups at coronal ($p = 0.088$), middle ($p = 0.427$) and apical ($p = 0.225$) thirds. Comparing 13.8% ClO_2 with 2.5% NaOCl, no significant difference was found between the two groups at coronal ($p = 0.324$), middle ($p = 0.733$) and apical ($p = 0.970$) thirds.

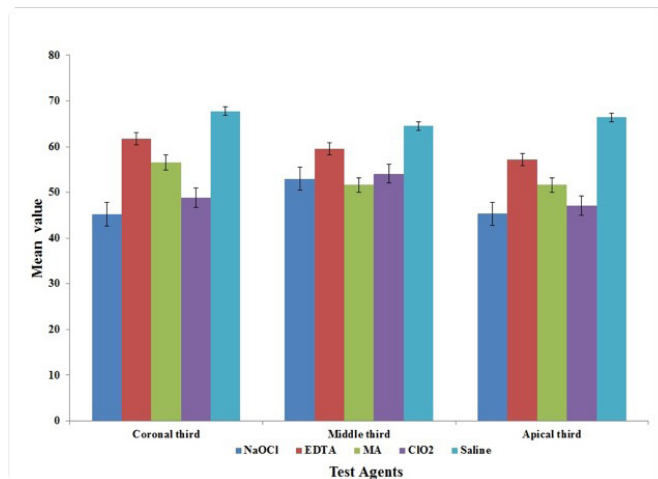


Figure 1: Mean microhardness values among the test agents in the coronal, middle and apical thirds of root canal dentin. (NaOCl= sodium hypochlorite; EDTA= ethylenediaminetetraacetic acid; MA= maleic acid; ClO_2 = chlorine dioxide)

When 7% MA was compared with 17% EDTA, there was no significant difference at the coronal ($p = 0.289$) and apical ($p = 0.226$) thirds. However, at the middle third 7% MA reduced the microhardness more than 17% EDTA ($p = 0.004$). On comparison of 7% MA with 2.5% NaOCl, there was no significant difference at the middle ($p = 0.325$) and apical ($p = 0.545$) thirds. However, in the coronal third, 2.5% NaOCl reduced the microhardness more than 7% MA ($p = 0.031$). When 17% EDTA was compared with 2.5% NaOCl, 17% EDTA reduced microhardness less compared to 2.5% NaOCl at coronal, middle and apical thirds ($p = 0.001$).

Surface roughness

Figure 2 shows the mean surface roughness among the different experimental groups. When the results of the surface roughness were analyzed, 7% MA produced maximum

surface roughness and the control group (saline) showed the lesser roughness. When 17% EDTA was compared with 2.5% NaOCl, there was no statistical significant difference between the two groups ($p = 0.821$). On comparison of 17% EDTA with 13.8% ClO_2 , 17% EDTA produced a rougher surface ($p = 0.049$). When 2.5% NaOCl was compared with ClO_2 , there was significant difference in which 2.5% NaOCl produced more rough surface ($p = 0.05$). Figure 3 demonstrates the 3D AFM images of dentin surfaces after treating with 7% MA, 17% EDTA, 13.8% ClO_2 , 2.5% NaOCl and 0.9% saline. It can be observed that, in MA group, the samples had more irregular surface, exhibiting numerous deeper peaks and valleys, suggesting a more evident rough surface. Similarly, in EDTA, NaOCl and ClO_2 groups, there was rise in the peaks and deeper valleys which were comparatively less than MA group. In saline group, the peaks and valleys were minimal due to the smooth surface texture of root canal dentin.

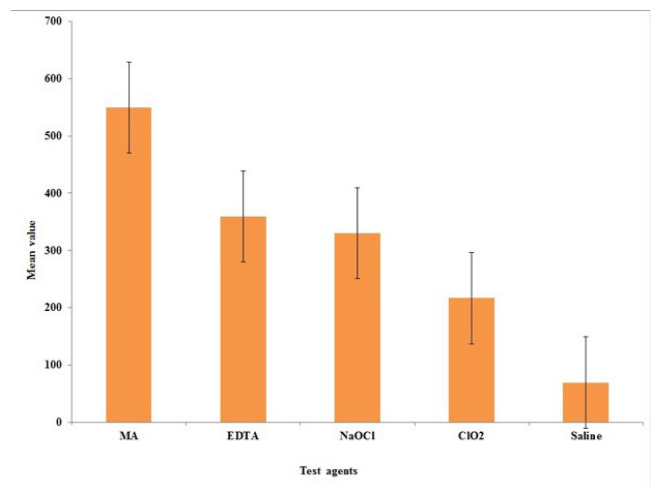


Figure 2: Mean surface roughness values among the test agents in the coronal, middle and apical thirds of root canal dentin. (NaOCl= sodium hypochlorite; EDTA= ethylenediaminetetraacetic acid; MA= maleic acid; ClO_2 = chlorine dioxide)

DISCUSSION

The measurement of the hardness of dental substrates and related materials is one of the simplest nondestructive mechanical characterization methods. Microhardness testing provides a numerical value that allows comparison between materials submitted to the penetration of a specific indenter. The hardness value depends on various factors. Thus, the hardness value obtained cannot be considered as a basic property of the material, rather an indication of its behavior in a specific condition. Panighi and G Sell²⁹ reported a positive correlation between microhardness and the mineral content of the tooth. Therefore, determination of microhardness can provide valuable evidence of mineral loss or gain in the dental hard tissue,³⁰ with special regard here, to the effects of irrigating solutions on root dentin hardness.

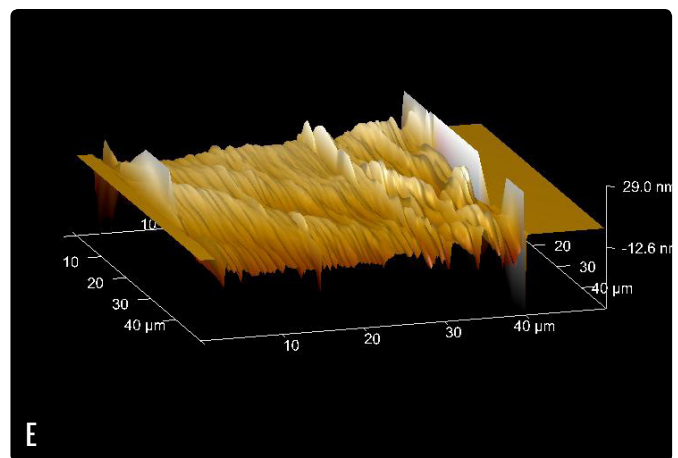
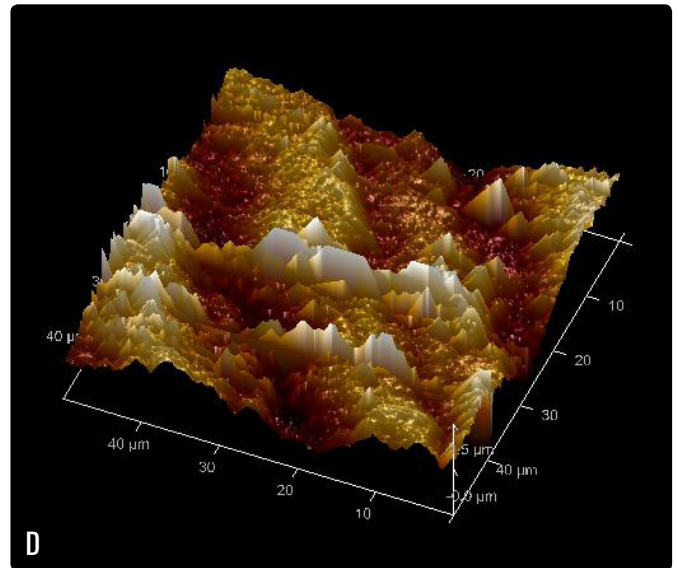
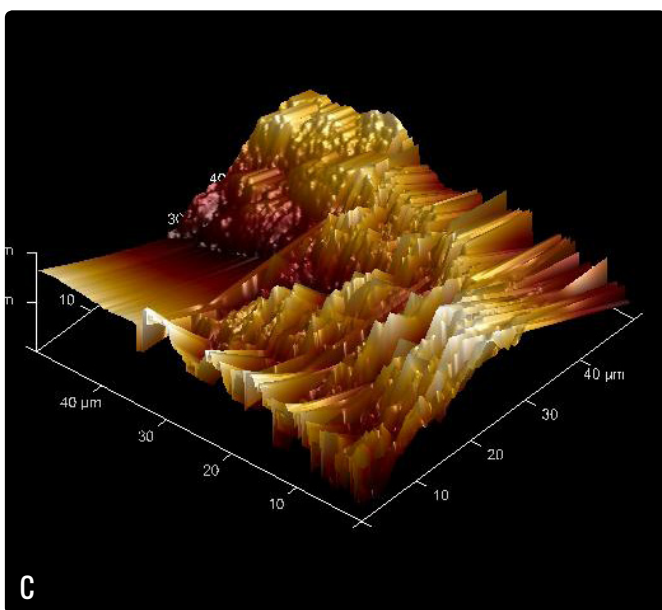
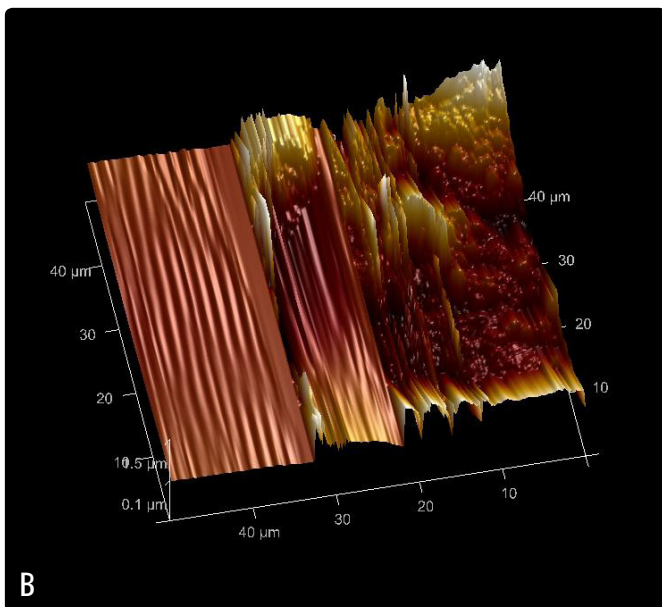
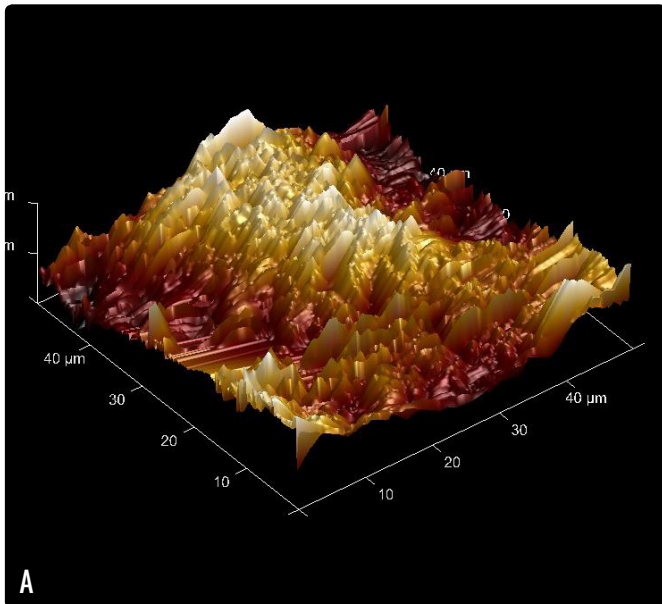


Figure 3: Three dimensional atomic force microscopic images of root canal dentin surfaces after treatment with the test agents. Group A had maximum rough surface compared to other groups. Group E showed minimal surface roughness. [A] 7% maleic acid; [B] 13.8% chlorine dioxide; [C] 17% ethylenediaminetetraacetic acid; [D] 2.5% sodium hypochlorite; [E] 0.9% Saline

The methodology used in the current investigation represents an approximation of the clinical scenario where irrigation forces and physical properties of fluid dynamics may intervene, however, the standardization of testing irrigation solutions favors the used of root halves specimens.³¹ The results of the present study indicated significant differences in the reduction of microhardness between all the experimental groups at different thirds of the root canal system. Saline, which was used as a negative control, was found to have a minimal effect on the microhardness of root canal dentin compared with other test agents. Saline does not have any chelating or demineralizing effect on the root dentin and this finding corroborates with a previous study from our group.²⁷

When 7% MA and 17% EDTA was compared, there was no significant difference in surface hardness since, both 7% MA and 17% EDTA solutions have shown to decalcify the mineral content of root canal dentin, subsequently reducing the

microhardness. This finding is in agreement with previous work.²⁷ Since 7% MA has demonstrated superior smear layer removal ability than 17% EDTA, and also a non-significant difference between 7% MA and 17% EDTA in reduction of root canal dentin microhardness exists, MA can be used in place of EDTA. However, when 17% EDTA was compared with 13.8% ClO₂ and 2.5% NaOCl, the former reduced the microhardness less. This may be because both 13.8% ClO₂ and 2.5% NaOCl have a unique way of reducing the microhardness of root canal dentin. It has been already reported that, use of NaOCl alone can induce a significant reduction in dentin microhardness.²⁸ This may be because NaOCl causes dissolution of the collagen fibrils or the collagen mineral bond.³² Further, NaOCl does not only affect the organic matrix of the root canal dentin, but also the inorganic content that represents the lattice of the tooth structure.³³ Microhardness testing on a heterogenous material like dentin varies due to differences in tubular density from apical to coronal dentin, which may lead to deviations in the results as seen in the present study. In the present study, to measure the Knoop hardness values for dentin, indentations were made from coronal, middle and apical thirds of the root canal and were performed at 0.5 mm distance from the root canal walls for standardization. Nano-hardness studies are less reliable due to the possibility to indent specific areas such peritubular and intertubular dentine. Microhardness tests have proven to provide more normally distributed data.

Dentin hardness is related to location and its value decreases as the indentation tested are made closer to the pulp (34). Pashley *et al.*³⁴ reported that the microhardness of dentin decreased when dentin was tested from superficial to deep regions. He also reported an inverse correlation between dentin microhardness and tubular density. In addition to tubular density, the contact time of the irrigation solution needs to be considered as another determinant in the post treatment microhardness values of dentin. Currently, there is no consensus on the optimal time an irrigating agent must be in contact within the root canal system. However, 1 minute time interval was chosen here because studies have reported that EDTA when used for more than one minute causes over-chelation with subsequent funnel shape erosion of dentinal tubules, thus reducing the dentin micro hardness.^{35,36} Similarly no guideline exists regarding the exact amount of irrigating solutions to be used clinically. 5 ml of irrigation solution was chosen in this study as proposed by Mello *et al.*³⁷. The relative softening effect on the dentinal walls exerted by these chemicals irrigants could be of clinical benefit since it permits rapid preparation and facilitates negotiation of tight root canals due to lubricant and chelating activity.³⁸ Still, these alterations may also affect the sealing ability and adhesion of sealers and adhesives to the root dentin.³⁹

The results of the present study for surface roughness indicated that, 7% MA produced the roughest surface of the root canal dentin. This could be due to the higher demineralizing

ability of 7% MA compared to other test agents. This finding is in accordance with a previous study²⁷ which showed that, 7% MA produces increased roughness when compared to 17% EDTA. An increase in the surface roughness could be of clinical benefit in restorative dentistry/endodontics because of micromechanical bonding of adhesive restorative materials and root canal resin sealers that require the presence of irregularities on the surface of the adherend into which the adhesive can penetrate.

Surface roughness can be measured by various methods like atomic force microscopy (AFM) and profilometry. However, in this study AFM was used because it possesses several advantages: samples require little or no preparation, single samples can be mapped several times and image properties can be obtained quantitatively by high resolution three dimensional images.⁴⁰

Further studies should investigate the effect of combined irrigation regimens where different solutions are used alternatively. Of interest will be also to determine the adhesion of endodontic sealer to different dentinal substrates prepared by different irrigation systems.

CONCLUSION

Based on the results obtained and experimental conditions of the present study, it can be concluded that 13.8% ClO₂ and 2.5% NaOCl reduced microhardness more than 17% EDTA. There was no significant difference between other experimental groups. 7% maleic acid produced maximum surface roughness compared to other test agents. Saline which was used as a negative control did not alter the radicular dentin microhardness and surface roughness.

MANUFACTURERS' DETAILS

- Sodium azide, Sigma Chemical Co, St. Louis, MO
- Diamond point, Diatec, Coltene AG, Switzerland
- Barbed broach, Mani Inc, Tochigi Ken, Japan
- Diamond disc, Horico, Germany
- Alumina suspension, Ultra-Sol R, Eminess. Tec.Inc.Monroe, NC
- Chlorine dioxide, BioClenz, Frontier Pharmaceutical, Inc, Melville, NY, USA
- 17% EDTA, Merck, Dermstadt, Germany
- 7% Maleic acid, KMC Pharmacy, India
- 2.5% Sodium hypochlorite, KMC Pharmacy, India
- Knoop hardness tester, Matsuzawa Co. Ltd, Akita, Japan
- Atomic force microscope, Veeco, Santa Barbara, CA

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