Comparative evaluation of decalcifying agents for dissolution of pulp stones: An *in vitro* study

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**Abstract**

**Background:** Despite constant advances in science, obscurity remains in the efficient removal of pulp stones to aid in successful root canal treatment. In this context, chemical means of dissolving pulp stones were explored.

**Aim:** The aim of this study is to evaluate and to compare the efficacy of decalcifying agents on the dissolution of pulp stones.

**Materials and Methods:** The study was divided into two groups for pulp stone analysis (21 samples) and dentin analysis (54 samples). Twenty-one pulp stones from patients aged 18–70 who underwent root canal treatment were collected and divided into three subgroups (n = 7) randomly. They were subjected to chemical treatment in a labeled glass container with 5 ml of the respective chemical agents, such as 17% ethylenediaminetetraacetic acid solution (positive control), no treatment (negative control), and newly developed Physiological Simulated Decalcifying Agent (PSDA). At the end of the study period (24 h), the samples were removed, rinsed with deionized water, and subjected to physical analysis, scanning electron microscopy (SEM), and Energy –dispersive X-ray spectroscopy (EDS) analysis. Under dentin analysis, 54 maxillary premolars scheduled for orthodontic extraction without caries or extensive restorations were selected, following which 2-mm thick transverse dentinal sections at the cementoenamel junction level were obtained and randomly divided into two groups for SEM (n = 21) and microhardness analysis (n = 33). The samples were subjected to respective chemical treatment groups similar to pulp stones for 24 h and analyzed using SEM, EDS, and microhardness analysis.

**Results:** Postchemical treatment with the newly developed decalcifying solution, the pulp stones showed the absence of nodular crystallites and surface softening under SEM and a decrease in the calcium level under EDS analysis. Concerning the microhardness of dentin, no significant changes could be observed.

**Conclusion:** The newly explored PSDA was found to be efficacious in the decalcification of pulp stones at a clinically relevant time of 24 h, without significantly affecting the structural integrity and the hardness values of dentin.

**Keywords:** Chelating agents; dentin microhardness; pulp canal obliteration; pulp stones

**INTRODUCTION**

Pulp stones are nodular calcified masses that are found either in the coronal or root portion of the pulp. They are usually oval or round shaped and can also be irregular. A single tooth may have 1 or 12 or even more stones with varying sizes ranging from 50 μm to larger masses that can occlude the pulp space as well. The prevalence can be close to 100% with varying sizes, particularly if associated with an increase in age and physiological factors. Recent systematic reviews and meta-analysis studies stated that the prevalence of pulp stones was found to be 36.53%. The etiology of pulp stones remains to be obscure. However, local factors, such as trauma, aging, caries, restorations, periodontal disease, orthodontic treatment, and...
systemic conditions, such as dentin dysplasia, cardiac disease, and kidney diseases, happen to be potential risk factors for the occurrence of pulp stones. They have structural and chemical properties similar to dentin and are mainly composed of calcium (32.1%) and phosphorous (14.7%).

Thorough knowledge regarding the clinical challenges posed by pulp stones can help the dental practitioner in achieving successful treatment outcomes. If larger, they alter the internal anatomy of the tooth, deflecting the tip of the instruments, and hindering the root canal instrumentation, leading to inadequate removal of the pulp tissue. They also block the access to root canal orifice leading to technical failures such as gouging of the pulp chamber, missed canals, perforation, and instrument separation, which results in poorer root canal treatment outcomes.

The removal of pulp stones from the pulp chamber is a difficult, laborious, and time-consuming process that not only requires skill and dexterity but also expensive equipment such as ultrasonic tips, C pilot files, and magnification devices. However, these procedures are suitable only for loose calcifications and lead to excessive loss of tooth structure and a higher failure rate. Based on this thought, chemical means for dissolving the pulp stones were explored to reduce the above-discussed limitations caused by instrumental methods. A newly developed solution “Physiological Simulated Decalcifying Agent (PSDA)” with a pH of 2.5 was formulated to dissolve the pulp stones. Hence, an in vitro evaluation study was performed to check the efficacy of the newly developed chemical reagent on the dissolution of pulp stones and its effect on the structural integrity of dentin.

MATERIALS AND METHODS

Specimen selection and preparation
Ethical clearance was obtained from the Institutional Ethical Committee with reference no. EC-2019/PG/017. Pulp stones were retrieved from patients aged 18 to 70 who underwent endodontic treatment in the Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences. The presence of pulp stone was confirmed radiographically. Only intact pulp stone of size equal to or more than 1 mm × 2 mm was included, and friable pulp stones were discarded. Pulp stones, as and when collected, were rinsed with phosphate-buffered saline (pH 7.4) to remove any exogenous material present and dried out as shown in Figure 2c. The samples were then subjected to respective chemical reagents immediately to avoid any physical changes in storage.

Preparation of dentin disks
Fifty-four intact, mature maxillary premolars extracted for orthodontic purposes were selected within 6 months of extraction and stored in 0.1% thymol solution at room temperature. The maxillary premolars with root cracks and which were endodontically treated were excluded from the study. Each tooth was mounted in a die stone block for dentin disk preparation. The occlusal enamel of each tooth specimen was removed till the dentin surface was exposed using a high-speed handpiece with a wheel-shaped diamond bur. The tooth specimens with the exposed dentin were cut horizontally to obtain 2-mm thick dentin sections from the cemento-enamel junction using a low-speed saw (Minitom, Struers, and Copenhagen, Denmark) under water cooling. These dentin sections were polished using 120 grit Si-C paper to obtain a flat surface. The specimens were then ultrasonicated in distilled water for 10 min using an ultrasonic bath (FS20, Fisher Scientific Co., Pittsburgh, PA, USA) to remove the smear layer so caused by the polishing process, as shown in Figure 1a-c.

Grouping method

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Following this, the dentin sections were subjected to chemical tests.

Grouping method

Pulp stones and dentin—qualitative analysis
A power analysis was established by G*power, version 3.0.1 (Franz Faul, Universität, Kiel, Germany). A sample size of 42 subjects (21 in each group—pulp stone and dentin) with \( n = 7 \) in each subgroup would yield 80% power to detect significant differences, with an effect size of 0.8 and significance level at 0.05.

- Subgroup 1: Ethylenediaminetetraacetic acid (EDTA)
- Subgroup 2: No treatment
- Subgroup 3: PSDA solution.

Dentin—quantitative analysis
The sample size was calculated using the formula:
n = 2 \left(\frac{Z^2(S + Z)}{M_1 - M_2}\right)^2

Where, M1 = 36.7°, M2 = 43.5°, Pooled standard deviation (S) = 5.21, Z1 = 1.64 (90% confidence interval), Z2 = 1.28 (90% power). Substituting the above values in the formula, the sample size was found to be 11. Since there are three groups, the total sample size was estimated to be 11 \times 3 = 33.\[13]

- Subgroup 1: EDTA
- Subgroup 2: No treatment
- Subgroup 3: PSDA solution.

**Formulation of physiological-simulated decalcifying agent**

- Reagent A: Accurately weighted 1.225 g of potassium hydrogen phthalate (PHT) was dissolved and suitably made up the volume of 30 ml with demineralized water
- Reagent B: 0.34 ml of concentrated hydrochloric acid was diluted to 40 ml with demineralized water
- Reagent C: 2 ml of dimethyl Sulfoxide.

**Methodology**

Mixing 25 ml of Reagent A (0.2 M) with 37 ml of Reagent B (0.1 M) in a glass beaker using a propeller stirrer at 200 rpm for 10 min. A volume of 2 ml of Reagent C was added to the above solution, and volume was diluted to 90 ml using demineralized water and further mixed for another 5 min. The final volume make was done to 100 ml using demineralized water and homogenized for 5 min using ultrasonic water bath.

The preparation was made by slightly modifying the formula ingredients and quantities as stated in the Indian Pharmacopoeia-1996.

**Chemical treatment of pulp stones and dentin**

The pulp stones and dentin sections were randomized into two control groups and one experimental group (Group 1: positive control – 17% EDTA solution, Group 2: negative control, and Group 3: PSDA solution). They were immersed in a labeled glass container with 5 ml of the chemical agents to the groups allocated. The samples were maintained at 37°C for 24 h. Following this, the samples were rinsed with deionized water, dried, and subjected to further analysis.\[11]

**Scanning electron microscopy and Energy–dispersive X-ray spectroscopy (EDS) analysis**

Following chemical treatment, the specimens were fixed in stubs, and each sample was sputter coated with a 5-nm layer of gold (Bal-tec SCD 500, Bal-tec AG, Balzers, Liechtenstein).\[10] A scanning electron microscope (EDAX, Ametek, Inc. USA) was used to observe the microstructure of pulp stone and dentin. Images at a constant magnification of 75.00 KX, 50.00 KX, 25.00 KX, and 100.00 KX were obtained. Energy–dispersive X-ray spectroscopy (EDS) analysis was done to observe the chemical composition of the dentin and pulp stone postchemical treatment.\[11,10]

**Microhardness testing**

Microhardness testing was done using Highwood DMH7, Japan (Model: HWMMT– X7) microhardness intender. Samples were slightly polished with 150 grit sandpaper to make them flat. A load of 50 g for 10s was applied. Indentations at two different points were made with a diamond indenter, in each dentin section sample and 11 samples of each group were tested.\[12]

The data were entered in the excel spreadsheet and analyzed using SPSS version 2.0 (IBM® SPSS® [IBM corp. Armonk, NY, USA released 2011]). Descriptive statistics of the explanatory and outcome variables were calculated by the median, interquartile range, and P value. Comparison of calcium, phosphorous, and microhardness values were made using Kruskal–Wallis test and intergroup using post hoc Mann–Whitney.

**RESULTS**

Physical analysis of the pulp stone postchemical treatment was done to check the endpoint of decalcification through a probing technique done mechanically by running a sharp probe into the specimen and measurement of weight using a precision weighing balance. The weight of the pulp stone before any chemical treatment was measured to be (0.3 mg) using a precision weighing balance (ACCULAB, Massachusetts, USA). Probing the pulp stone subjected to 17% EDTA solution with a needle was suggestive of incomplete decalcification even at the end of the study (24 h). However, after treatment with PSDA solution, the weight got decreased up to (0.2 mg), and the sample was found to be soft and fragile, as shown in Figure 2a and b.

**Scanning electron microscopy analysis of pulp stones**

Representative scanning electron microscopy images (SEM) of the samples are shown in Figures 3-5. Before any chemical treatment, the pulp stones displayed a rough, heterogeneous, and irregular structure, with the crystallites being closely packed on the surface, as shown in Figure 3a-d. Following treatment with EDTA, there was a loss of crystallites and surface softening due to the decalcification effect, as shown in Figure 4a-d. After treatment with the newly developed solution (PSDA), the surface topography of the pulp stone was found to be smooth as the internal surface was traced through higher magnification at 25.00KX substantiating the effect of decalcification, as shown in Figure 5a-d.
Scanning electron microscopy analysis of dentin

Representative images of SEM of dentin are shown in Figure 6. Before any chemical treatment, the dentinal tubule openings were smaller, as exhibited in Figure 6a and b. Following treatment with EDTA, the tubules were open and enlarged due to decalcification along with the deterioration of the dentinal surface, as shown in Figure 6c and d. After treatment with PSDA solution, the dentinal tubules were wide and open. The peritubular dentin and intertubular dentin areas were found to be covered with particulate residues along with calcific deposits blocking the dentinal tubule orifices as well as indicated in Figure 6e and f.
Energy –dispersive X-ray spectroscopy (EDS) analysis

Following chemical treatment of pulp stone and dentin with EDTA, there was greater reduction in the calcium and phosphorous level when compared to chemical treatment with PSDA group as shown in Tables 1 and 2.

Vickers microhardness test

EDTA caused a maximum reduction of dentin microhardness (4.50). However, there was no statistically significant difference between PSDA (24.8) and no treatment[14] as shown by post hoc Mann–Whitney test, as shown in Table 3.

DISCUSSION

Pulp stones are primarily a physiological manifestation that might increase in number or size due to local or systemic factors.[1,4] These pulp stones might differ in sizes ranging from microscopic particles to larger masses such that they nearly obliterate the pulp chamber, influencing the outcome of root canal treatment.[7] Despite constant advances in science, obscurity remains in the efficient removal of pulp stones to aid in successful root canal treatment. Although various instrumentation techniques have been described in the literature, the effectiveness of these techniques is hampered by the large size and attachment of pulp stones to dentin, leading to potential complications such as weakening of tooth structure or perforation.[15,16]

Hence, the current study is innovated by refining its outcome to understand the chemical means of dissolution of pulp stones to achieve a more qualitative outcome. A pilot study was done with various decalcifying agents like 5%, 10% nitric acid, and 5% formic acid, 1% acetic acid,[13,17,18] potassium citrate, magnesium citrate,[19] and Udiliv gel.[20] However, these agents demonstrated inadequate as well as a longer period for decalcification of pulp stones. This consecutive failure stimulated our thought process of developing a new decalcifying agent to add up to the contribution in this field.

The three reagents of PSDA were chosen based on the following criteria that it must decalcify at a reasonable speed, ensure complete removal of calcium, and cause minimal damage to surrounding tissues.[13,17,18] The solution majorly contained hydrochloric acid – an agent commonly used for decalcification that produced better results when compared to formic acid and nitric acid. It has also got excellent soft- and hard-tissue integrity.[13,17,18,21] Potassium hydrogen phthalate – an acidic salt compound that acts as a buffer and stabilizes the pH of the reagents,[22] and dimethyl sulfoxide – an inert solvent which acts as a penetration enhancer. It is also shown to improve the immediate and long-term bond strength of dentin.[14,23-25] To the best of our knowledge, this chemical reagent has never been formulated or used in any of the investigations conducted before to remove the pulp stones and hence had been applied for an Indian Patent (Provisional Application Number-202041055629).
Through the SEM and EDS results obtained, the chemical reagent developed was found to be efficacious in decalcifying the pulp stones on a par with EDTA. This may be attributed to the presence of hydrochloric acid present in the chemical reagent, which caused rapid decalcification (loss of calcium) following chemical treatment. [13] Due to the discrete differences in the level of crystallinity between the pulp stone and dentin, the action of PSDA was more targeted toward the pulp stone than dentin. [1,4,26]

Since it is a solution prepared in an indigenous manner, it gave excellent results on the decalcification of pulp stones after 24 h. The low-HCL concentration and shorter contact time of PSDA solution did not result in any adverse effects on the dentin, which is validated through the Vickers microhardness test. However, residual calcific deposits were found to be covering the dentinal tubules due to the low viscosity of the reagent.

In the comparison of the Vickers microhardness using the Kruskal–Wallis test, EDTA caused the maximum reduction of dentin microhardness due to its chelating property. [27–29] However, there was no statistically significant difference in the microhardness of dentin between the PSDA and negative control group. Although this is considered to be a clinical advantage, attention should be paid regarding the contact time of the solution, [29] and accordingly, further studies can be performed to investigate the effect of PSDA solution on the penetration of dentinal tubules and fracture resistance of dentin postchemical treatment.

Hence, this newly developed chemical solution figured as an excellent decalcifying reagent and a suitable alternative to EDTA while preserving the structural integrity and microhardness of the surrounding dentin. This innovation can be used more effectively to support the commercialization of this decalcifying agent and can be used while encountering patients with attached pulp stones. However, further planned in vivo studies would substantiate the results in the mere future.

**CONCLUSION**

The newly developed PSDA was found to be effective in the decalcification of pulp stones in 24 h when compared to EDTA, which takes a longer time for decalcification and without any deleterious effects on the dentin. In the clinical scenario, the commercialization of this decalcifying agent would aid the clinician in the easy and precise removal of pulp stones using hand instruments after 24 h, unlike the conventional instrumentation techniques, resulting in potential complications such as weakening of tooth structure or perforation.

| Table 1: Comparison of the calcium among the groups using Kruskal–Wallis |
|---------------------------------|----------|----------|-----------|-------|----------------|---------|
|                                  | n    | Minimum | Maximum | Median | IQR   | Kruskal–Wallis | P       |
| Pulp stone                       |      |         |         |       |       |           |         |
| EDTA                             | 7    | 0.00    | 25.85   | 18.4  | 20.84 | 12.47     | 0.002*  |
| No treatment                     | 7    | 25.84   | 40.54   | 32.89 | 7.92   |           |         |
| PSDA                             | 7    | 0.00    | 27.85   | 19.6  | 15.83 |           |         |
| Dentin                           |      |         |         |       |       |           |         |
| EDTA                             | 7    | 2.54    | 14.7    | 11.8  | 4.20  | 11.51     | 0.003*  |
| No treatment                     | 7    | 15.77   | 20.50   | 18.54 | 3.7    |           |         |
| PSDA                             | 7    | 10.54   | 19.69   | 15.42 | 8.06   |           |         |

IQR: Interquartile range, PSDA: Physiological-simulated decalcifying agent, EDTA: Ethylenediaminetetraacetic acid, *P<0.05 denotes significance

| Table 2: Comparison of the phosphorous among the groups using Kruskal–Wallis |
|---------------------------------|----------|----------|-----------|-------|----------------|---------|
|                                  | n    | Minimum | Maximum | Median | IQR   | Kruskal–Wallis | P       |
| Pulp stone                       |      |         |         |       |       |           |         |
| EDTA                             | 7    | 0.00    | 5.40    | 3.4   | 4.30  | 11.83     | 0.003*  |
| No treatment                     | 7    | 9.4     | 15.3    | 10.8  | 4.4   |           |         |
| PSDA                             | 7    | 0.00    | 14.6    | 5.25  | 7.30  |           |         |
| Dentin                           |      |         |         |       |       |           |         |
| EDTA                             | 7    | 0.46    | 11.65   | 6.8   | 6.24  | 5.92      | 0.052   |
| No treatment                     | 7    | 4.9     | 14.8    | 12.85 | 5     |           |         |
| PSDA                             | 7    | 5.30    | 12.13   | 7.6   | 4.20  |           |         |

IQR: Interquartile range, PSDA: Physiological-simulated decalcifying agent, EDTA: Ethylenediaminetetraacetic acid, *P<0.05 denotes significance

| Table 3: Comparison of the Vickers hardness test among the groups using Kruskal–Wallis |
|---------------------------------|----------|----------|-----------|-------|----------------|---------|
|                                  | n    | Minimum | Maximum | Median | IQR   | Kruskal–Wallis | P       |
| EDTA                             | 11   | 2.60    | 33.40   | 4.50  | 21.40 | 6.38        | 0.041*  |
| No treatment                     | 11   | 14.20   | 35      | 22    | 11.1  |           |         |
| PSDA                             | 11   | 14.40   | 47.20   | 24.8  | 17.8  |           |         |

IQR: Interquartile range, PSDA: Physiological-simulated decalcifying agent, EDTA: Ethylenediaminetetraacetic acid, *P<0.05 denotes significance
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Conflicts of interest
There are no conflicts of interest.

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