



Clearing Technique: Gateway to Demystifying Pulp Space Morphology

Rashmi Bansal^{1*}, Sapna Hegde² and Madhusudan Astekar³

¹PhD Scholar, Department of Dentistry, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India

²Professor, Department of Pedodontics and Preventive Dentistry, Pacific Dental College and Hospital, Udaipur, Rajasthan India

³Professor and Head, Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Bareilly, Uttar Pradesh, India

***Corresponding Author:** Rashmi Bansal, PhD Scholar, Department of Dentistry, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

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Abstract

Clearing technique is a simple and inexpensive technique for *in vitro* examination of root canal systems. The technique consists of decalcification with nitric acid, dehydration with alcohol, and clearing with methyl salicylate. It renders the teeth transparent. Knowledge obtainable using these transparent teeth permits an increased appreciation of the anatomy of the root canal system. This technique gives a three dimensional view of the pulp cavity in relation to the exterior of the tooth. Hence variations of root canal morphology in different teeth in different population can be evaluated by this technique.

Keywords: Canal Staining; Clearing Technique; Decalcification; Root Canal Morphology; Transparent tooth

Introduction

Clearing technique is a procedure routinely employed in histological processing to examine the internal anatomy of calcified tissues. In this dehydrated tissue is made ready for paraffin infiltration by immersion in wax solvent. By this tissue becomes transparent. This property was used by Okumura, *et al.* [1]. for the first time to make teeth transparent. Later it was modified by Tagger, *et al* [2]. Robertson [3] utilized this technique to observe root canal morphology of teeth.

The process of making tooth transparent involves many physical and chemical changes. The inorganic constituents of the tooth are first dissolved by decalcification and further water, air and lipid components are removed by dehydration [4]. Subsequent immersion in the clearing agents make tooth transparent by taking up its refractive index. Clearing agents are volatile in nature hence once the transparent tooth is taken out of the clearing agent, the solvent evaporates, and the tooth is invaded by air and moisture again, hence opacity of sample is reversed [5]. Time taken for tooth to become transparent in clearing agent is directly proportional to the time taken for it to become opaque, when it is out of clearing agent [6].

Transparent tooth model developed after clearing technique can be used to obtain information on various aspects of endodontic treatment like;

- I. Root canal configuration [4,5].
- II. Canal instrumentation techniques [2,6].
- III. Effect of post design and its influence on root fracture [7].
- IV. Penetration of human saliva through dentinal tubules [8].
- V. Sealer placement techniques in curved canals [9].
- VI. Microleakage of root canal sealers [10,11].
- VII. For obtaining information on the quality of root canal obturation [3,12-15].

Apart from clearing technique, there are various other techniques like cone beam computed tomography CBCT, Spiral CT, and sectioning etc. to visualize root canal morphology. Out of these techniques clearing technique is more accepted as - it is simple, little equipment and space is required and entire procedure is completed in 5 days. It gives three dimensional view of the pulp cavity in relation to the exterior of the teeth. In this technique it is not necessary to gain an access into the specimen with instruments,

thus original form and relationship of the canals are maintained [3]. Even minute structural details can be visible clearly with rare chance of sample distortion during preparation.

There are certain limitations to this technique also [9];

- I. Clearing technique remains useful only as a teaching research tool with little or no clinical applicability.
- II. Incomplete dehydration will lead the teeth with opaque areas. This is correctable by additional dehydration in 100% ethyl alcohol.
- III. Development of opacity after air drying. This is readily reversible by immersion in clearing agent.

In the past researchers have used many different materials to decalcify and dehydrate teeth and various clearing and storage agents have been used.

Demineralizing agents

They are used to remove calcified tissue by immersing in acidic solutions. Various demineralizing agents indicated for this purpose are;

- a. Nitric acid (5-11%) [2,5,6,8,16].
- b. Formic acid (20%) [17].
- c. Hydrochloric acid (5%) [4].
- d. Solution of ion exchange resin and formic acid (40%) [7].

Shrinkage of organic tooth tissue could occur during the demineralization process. This phenomenon may be avoided if a weak concentration of acid is used [3].

Agents used for dehydration [10]

Ascending gradient of ethyl alcohol is used to remove air and water from decalcified tissue. Various proposed series of concentration of ethyl alcohol are;

- o 25%,50%, 70%,90%,95% and 100% (30 min passage each)
- o 80%,90%, and 100%

Dyes to visualize morphology of root canal

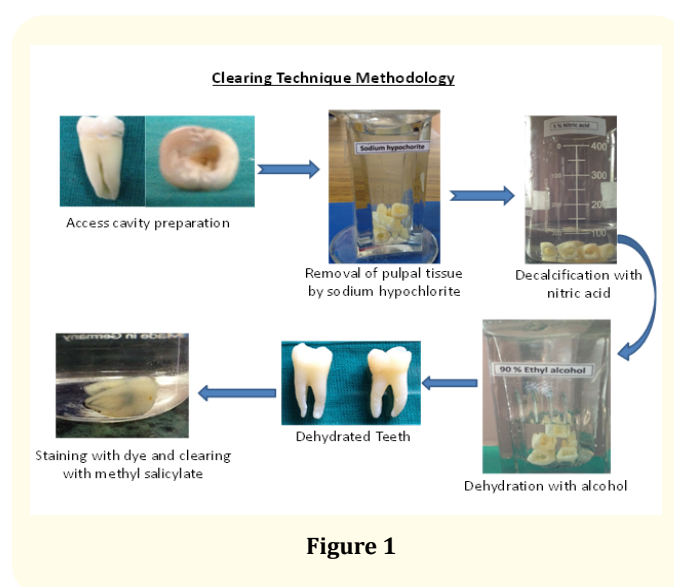
Various dyes are indicated to visualize the root canal morphology. Both water based and oil based dyes are available. In clearing technique oil based dyes prove to be a better tool in demonstration of root canal anatomy, as they can flow into minute details. Indian ink which is oil based dye is one of the most commonly used dyes [9].

Clearing agent

Teeth are placed in clearing solution having same refractive index to make them transparent. Various clearing agents suggested are.

- o Xylene, Benzene, Methyl salicylate, Eugenol, Casting resin. Each solution has its own advantages like Eugenol maintains the transparency of the tooth for the longest period of time outside the clearing agent, which serves its utility in imparting a longer working. Xylene is more corrosive in nature. Methyl salicylate tolerates more water and is non-noxious.

Clearing technique involves five main basic steps (Figure 1).



Preparation of tooth [3]

Teeth are cleaned of tissue and debris. Access cavity is prepared on teeth using round diamond point number 2 and high speed handpiece. Orifices of the canals are evaluated using endodontic explorer under magnification using loupes and avoiding any alteration of the pulpal floor and the root canal anatomy. Developmental grooves are carefully explored for extra canals.

Removal of pulpal tissue [3]

To achieve clearing, specimens are stored in 5.25% sodium hypochlorite solution for 48 hours at room temperature (37°C) to dissolve the debris and the remaining pulp tissue. All specimens are then rinsed in running water for 4 hours to remove traces of sodium hypochlorite. Mechanical methods of pulp removal are avoided to preserve root canal morphology.

Decalcification [3]

Most commonly used acid in various studies is 5% nitric acid. The specimens are stored in 5% nitric acid solution for 2 to 3 days at room temperature, to achieve demineralization. The solution is stirred 3 to 4 times a day and replaced daily. Decalcification can be assessed by the insertion of paper pin in the crown and with the help of radiographs. After completion of decalcification the teeth are rinsed in running tap water for four hours to remove traces of nitric acid. Incomplete or uneven decalcification can be avoided by agitation of acid three times a day, having sufficient volume of acid, daily changes of acid solution and not stacking teeth during demineralization as demineralization occurs more rapidly at the top of the container than at the bottom. The decalcification can be accelerated by heating the acid solution or by using a more concentrated acid solution. Either method can be used but by accelerating decalcification shrinkage may increase or digestion of the organic component of the tooth is probable.

Dehydration [3]

Dehydration process consists of series of ethyl alcohol rinses. In most of studies it involves a series starting with 80% solution overnight followed by 90% solution for an hour and 100% ethyl alcohol rinses for an hour. At the end of dehydration, no opacity to be retained.

Injection of dye into root canal [3]

India ink is used in most of studies. It is injected into the pulp chamber with a number 27 gauge needle on a Luer – Lok plastic disposable syringe. The ink then is withdrawn through the canal system by applying negative pressure through apical end of the tooth with the use of the central suction system. The latter provide 25 mm of mercury vacuum. The appearance of the dye at the apical end indicate the end of the process. The excess of ink is then removed from the surface of the tooth with gauze soaked in 100% ethyl alcohol, and the tooth is returned to the methyl salicylate solution until needed.

Clearing of tooth [3]

The dehydrated teeth are then placed in clearing agent like methyl salicylate which makes teeth transparent after two hours. The cleared teeth are examined under stereomicroscope for evaluation. The specimens are returned to methyl salicylate solution until needed.

Discussion

The aim of decalcification is to remove calcium salts from mineralized tissue using chemical solutions like acids, while preserving the organic portions. This technique can be used in research work to study root canal morphology. The read out is simple and can be subjected to interpretation by direct observation under microscope. But it can be used in *in vitro* techniques only.

Conclusion

Clearing technique is simple and inexpensive technique to observe root canal morphology. Hence it can be used in various studies to evaluate root canal configurations in various teeth.

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