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A Comparative Study of Tooth Decalcification Using Conventional Acid Decalcification with Heat Induced Acid Decalcification Using Thermostatically Controlled Tissue Floatation Bath

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KEYWORDS

Nitric acid 5%, Formic Acid 5%, Ethylene diamine tetra acetic acid 14%, Conventional method, Tissue floatation bath method, time taken, discolouration, tearing and shrinkage, staining characteristics.

ABSTRACT:

Introduction: In order to examine the tooth it is softens by decalcification. To rapid the procedure temperature is increased by using tissue floatation bath (TFB).

Objectives: The aim of the study was to evaluate and compare the decalcification of teeth with three different decalcifying agents by two different methods with special attention to the duration of decalcification and by recording the staining efficacy of the pulpal tissue.

Methods: Sample size analysis was done by G*Power version 3.1 (Franz Faul, Universitat Kiel, Germany). In this study we use a sample of 60 freshly extracted tooth and compared the time taken, discolouration, tearing – Shrinkage and staining efficiency by using Nitric acid 5%, Formic acid 5% and Ethylene diamine tetra acetic acid (EDTA) 14% using conventional method and tissue floatation bath method (45° Celcius). The completion of decalcification was assessed by calcium oxalate test. Decalcified tooth undergoes tissue processing such as fixation, dehydration, clearing, infiltration and embedding. Hematoxylin and eosin stains were used.

Results: Nitric acid 5 % using TFB decalcifies the tooth rapidly when compared to other decalcifying procedures. Nitric acid 5% produces more discolouration of tooth. EDTA 14 % produces more tearing and shrinkage of tooth. Tooth decalcified by Nitric acid 5% using TFB shows better staining characteristics when compared to other decalcifying procedures.

Conclusions: In our study we use TFB because it is of rare method for decalcification of tooth, readily available, less expensive, compatible., fastens decalcification and improved the staining efficacy.

1. Introduction

Tooth tissue, composed of enamel, dentin, and cementum, is unique in its high mineralized matrix, primarily consisting of calcium, phosphorus, and biological apatite, making it chemically inert and challenging to study. Due to its physical hardness, it is necessary to soften tooth tissue by removing these minerals, a process known as "decalcification" or "demineralization."[1]

Decalcification, a critical process in histopathology, particularly in oral pathology, involves removing inorganic calcium from the organic collagen matrix, calcified cartilage, and surrounding tissues.[2] Various agents are used for decalcification, including strong inorganic acids, weak organic acids, and chelating agents like ethylene diamine tetraacetic acid (EDTA).[8] The efficiency of decalcification is influenced by factors such as the concentration of decalcifying agents, temperature, agitation, and suspension. These agents

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either form soluble calcium salts or chelate calcium ions, allowing the study of organic components that cannot be analyzed in ground sections.[3]

Decalcification enables the preparation of specimens soft enough to cut with a microtome knife, preventing damage to both the tissues and the knife. This process is crucial for examining the complex structures of the head and neck, where most soft tissues do not require prior treatment before tissue processing. In dental research, thin sections of teeth obtained through decalcification provide valuable insights into the histological observations of the pulp, dentin, and cementum.[3] Specifically, decalcification is essential for evaluating the pulpal soft tissue, which is often lost in ground sections. This evaluation is vital for assessing the biological response of the pulp to new restorative materials, diagnosing various bone and dental tissue pathologies (such as osteosarcoma, chondrosarcoma, Ewing's tumor, fibrosarcoma, spindle cell sarcoma, angiosarcoma, and chondroma), and understanding developmental processes related to teeth and pulpal tissue.[9]

The choice of decalcifying solutions depends on their speed, impact on teeth, and staining efficiency. Ideal agents should decalcify teeth rapidly while preserving soft tissue integrity and providing excellent staining quality. Although decalcification is a lengthy process that delays diagnosis, it remains essential in routine histopathological practice. To expedite decalcification, heat-induced methods are employed, including microwave, sonication, and electrolyte techniques.[4, 5, 6, 7, 8] However, the use of microwave ovens has drawbacks, such as being expensive, taking up significant laboratory space, and producing poor cytomorphological details. These issues can be mitigated by using a tissue floatation bath, which is less expensive, occupies less space, provides better cytomorphological records, and is commonly available in histopathological laboratories.

In conventional decalcification, teeth are processed at room temperature with regular solution changes until the endpoint is achieved. The tissue floatation bath technique involves intermittent placement of teeth in a bath maintained at 45°C, with decalcifying solutions changed regularly to expedite the process. This method allows for rapid decalcification while maintaining good cellular morphology.

In our study, we utilized the tissue floatation bath technique to rapidly decalcify teeth, ensuring good cellular morphology.[9,10] The study aimed to identify the ideal decalcifying solution based on rapid decalcification, minimal discoloration, tearing, and shrinkage of teeth, and effective staining in both conventional and tissue floatation bath methods using three different decalcifying solutions.

2. Objectives

The aim of the present study was to evaluate and compare the decalcification of teeth with three different decalcifying agents such as nitric acid 5% (HNO_3), formic acid 5% (CH_2O_2) and ethylene diamine tetra acetic acid 14% (EDTA) by conventional method and TFB with special attention to the duration of decalcification and by recording the staining efficacy of the pulpal tissue.

3. Methods

Prior to the commencement of the study, ethical approval was obtained from the institutional review board of Best Dental Science College & Hospital, Madurai, India.

Table 1. The groups in the study

1. CONVENTIONAL METHOD	**2. TFB METHOD**
1.a Nitric acid 5%	2.a Nitric acid 5%
1.b Formic acid 5%	2.b Formic acid 5%
1.c EDTA 14 %	2.c EDTA 14%

All reagents and chemicals used in the study were of analytical grade and used as purchased. Studies were done using the 60 freshly extracted teeth from the patient undergoing routine orthodontic treatment (maxilla or mandibular, first premolar or second premolar) from the department of oral and maxillofacial surgery. Teeth with caries, mechanical wearing and developmental disturbances were excluded. Sample is prepared by cutting the apex for about 2 mm for diffusion of fixative and for proper fixation in 10% formalin for twenty four hours.[11] . The sample is segregated into two study groups (conventional method and tissue floatation bath method) and three sub groups (5% nitric acid, 5% formic acid and

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14% Ethylene diamine tetra acetic acid - EDTA) (Table 1).[10] For Sample size analysis G*Power version 3.1 (Franz Faul, Universitat Kiel, Germany) was used a sample size of 10 in each group with the total sample size of 60.[12] Conventional decalcification method was done by hanging the tooth specimen in order to avoid contact that fastens decalcification. Change of acid was done in every twenty four hours where as in TFB method teeth decalcified by 45° Celsius. Change of acid done in every six hours in a beaker containing 100 ml of acid. (5% nitric acid, 5% formic acid or 14% ethylene di - amine tetra acetic acid). [13] Radiographic and chemical methods were used to assess the end point of decalcification. In Radiographic examination freshly extracted tooth appears radio opaque whereas decalcified tooth appears radiolucent due to disintegration of inorganic components (Calcium). In Chemical method : Calcium Oxalate test (Clayden 1952) was used The precipitation of the insoluble calcium hydroxide and calcium oxalate in acid solutions was used in the detection of the calcium.[14, 15.] Decalcified tooth undergoes tissue processing such as fixation, dehydration, clearing, infilteration and embedding. The microtomy was performed on paraffin wax-embedded decalcified teeth blocks using Microtome knives, disposable blades, paraffin section cutting. Stains such as Hematoxylin (Harry's haematoxylin) and eosin stains were used.[15, 16.]

4. Results

Figure 1 shows the histology and morphology of result of decalcification. Teeth decalcified by tissue floatation bath decalcifies rapidly when compared to the teeth decalcified by the conventional method**.** Teeth decalcified by nitric acid 5% decalcifies rapidly when compared to that of formic acid 5% and EDTA 14%. Teeth decalcified by nitric acid 5% in tissue floatation bath decalcifies rapidly (Mean value is 3.0 ± 0.4 days), where as the teeth decalcified using EDTA 14% in conventional method decalcifies slowely (mean value is 93.7 ± 1.0 days) when compared to other methods of decalcification (Fig. 2).

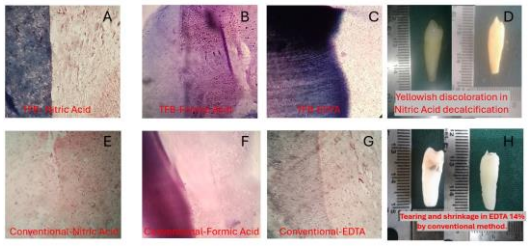

Fig 1. Histological Sections and decalcified teeth

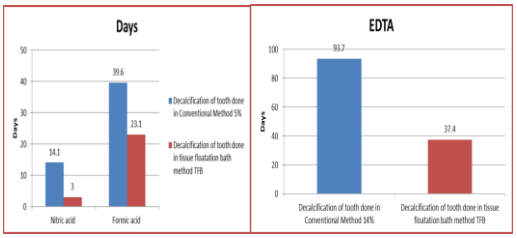

Fig 2. Graphical representation of number of days taken for decalcification using following acids by conventional and tissue floatation bath decalcification method.

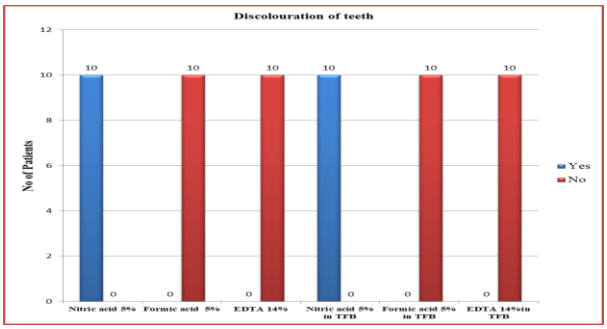

Fig 3. Graphical representation of discolouration of teeth in conventional and tissue floatation bath decalcification method.

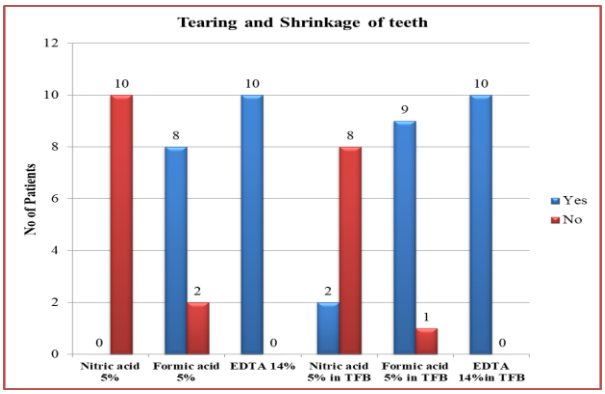

Fig 4: Graphical representation of tearing and shrinkage of teeth in conventional and tissue floatation bath method.

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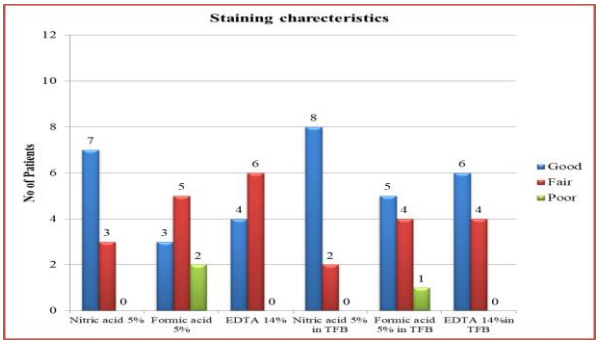

Fig 5. Graphical representation of staining characteristics of pulpal tissue in conventional and tissue floatation bath decalcification method.

For Macroscopic parameter - Nitric acid 5% discolours more when compared to that of formic acid 5% and EDTA 14%. Tearing and shrinkage of teeth - EDTA 14% produces more tearing and shrinkage of teeth when compared to that of nitric acid 5% and formic acid 5%. Teeth decalcified by EDTA 14% in TFB showed more tearing and shrinkage, where as teeth decalcified by nitric acid 5% using conventional method of showed less tearing and shrinkage (Fig. 3-4).

For staining charecteristics Nitric acid 5% showed better staining characteristics when compared to that of ethylene diamine tetra acetic acid 14% and formic acid 5%**.** Teeth decalcified by tissue floatation bath produced better staining characteristics when compared to that of teeth decalcified using conventional methods. Decalcification of teeth by nitric acid 5% in tissue floatation bath showed better staining characteristics when compared to that of all other acids and methods of decalcification. Formic acid 5% in conventional method showed the least staining characteristics when compared to that of all other acids and methods of decalcification (Fig. 5).

5. Discussion

The present study aimed to evaluate the efficiency and impact of different decalcifying agents and methods on the decalcification of teeth, focusing on decalcification speed, macroscopic parameters, tearing and shrinkage, and staining characteristics. The comparison between the conventional method and the tissue floatation bath (TFB) method, as well as the use of nitric acid, formic acid, and EDTA as decalcifying agents, provided valuable insights into optimizing the decalcification process for histopathological analysis.

The results demonstrate that the TFB method significantly accelerates the decalcification process compared to the conventional method. Specifically, teeth decalcified with 5% nitric acid in the TFB method showed the fastest decalcification time, with a mean value of 3.0 ± 0.4 days. In contrast, teeth decalcified with 14% EDTA using the conventional method exhibited the slowest decalcification time, with a mean value of 93.7 ± 1.0 days. These findings highlight the efficiency of the TFB method, particularly when using nitric acid, in rapidly decalcifying teeth. This accelerated process is beneficial in clinical settings where timely diagnosis is crucial.

In the present study, we found decalcification in TFB method four times faster than the conventional method for 5% nitric acid. For 5% formic acid and 14 % EDTA, it was twice faster in TFB method when compared to that of conventional method.by comparing studies done by Karpagaselvi Sanjai et al[2] , formalin- nitric acid solution took about 30 days for decalcification, 5% trichloro acetic (45 days), Perenyi's fluid took (55 days), 10 % formic acid (70 days), neutral EDTA (98 days) for decalcification. However in our study the time taken for decalcification using 5% nitric acid was around 14 days.Compared to other studies nitric acid 5% decalcifies rapidly because we use single rooted premolar, tooth is suspended to avoid contacts and acids were changed at regular intervels for 24 hours till the completion of decalcification. Decalcificaton by Nitric acid 5% in both TFB and Conventional method produces discolouration because nitric acid combines with oxygen to form nitric oxide which produces discolouration.[17, 18, 19.] Tooth decalcified by EDTA 14% and formic acid 5% shows more tearing and shrinkage when compared to that tooth decalcified by Nitric acid 5% because tissues which present in the acid for more amount of time produces more tearing and shrinkage.[13.] By comparing studies done by Ahmad Danish Rehan et al (2017) found that the tooth decalcification done in 5% nitric acid showed yellowish discolouration. Tooth decalcified by EDTA 14% and Formic acid 5% showed more tearing and shrinkage. In our study teeth decalcified by nitric acid 5% in TFB showed good staining characteristics followed by teeth decalcified in nitric acid 5% (conventional**)**, EDTA 14% in TFB method and EDTA 14% in conventional method. Teeth decalcified by formic acid 5% in conventional and TFB showed less

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staining efficacy when compared to all the other methods of decalcification. Since the fixation of pulp tissue was found to be inadequate it was difficult to evaluate the soft tissue integrity.[20, 21, 22.] By comparing the studies done by Archana Srinivasaiya et al (2016) found that tooth decalcified by 5% nitric acid using microwave method shows good staining charecteristics where as fair staining characteristics was shown by 5% and 7% formic acid and 7% trichloroacetic acid.[23] In our study we found that TFB method for decalcification was found to be faster when compared to other heat induced methods of decalcification also TFB is compatible, cost effective and readily available instrument in Pathological laboratory.[13]

Macroscopic examination revealed that 5% nitric acid caused more discoloration of the teeth compared to 5% formic acid and 14% EDTA. Discoloration can be a drawback, especially when assessing the structural integrity and aesthetic qualities of the decalcified specimens. However, the speed of decalcification with nitric acid may offset this disadvantage in scenarios where rapid processing is prioritized.

The study found that 14% EDTA caused more tearing and shrinkage of teeth compared to 5% nitric acid and 5% formic acid. Notably, teeth decalcified with 14% EDTA in the TFB method exhibited the highest degree of tearing and shrinkage, while those decalcified with 5% nitric acid using the conventional method showed the least. This suggests that while EDTA is effective in removing mineral content, it may compromise the structural integrity of the specimens. On the other hand, nitric acid, particularly in the conventional method, maintains better tissue integrity, reducing tearing and shrinkage.

The staining quality of decalcified tissues is crucial for subsequent histopathological examination. Nitric acid (5%) demonstrated superior staining characteristics compared to 14% EDTA and 5% formic acid. Furthermore, the TFB method produced better staining results than the conventional method. The combination of 5% nitric acid and the TFB method yielded the best staining characteristics among all tested conditions. This finding underscores the suitability of this combination for producing high-quality histological sections, facilitating accurate diagnosis and analysis.

Conclusion

Routine decalcification of teeth specimen is time consuming procedure in the histopathology labs, leads to undue delay in diagnosis and further delays the treatment process. heat induced decalcification methods were used to reduce the time taken for decalcification procedure. In our study we use tissue floatation bath (TFB) method at 45 °C for the first time in teeth decalcification. It reduces the time produces improved staining efficacy. It is an alternative for other heat induced method of decalcification. This will definitely reduce the waiting period for histo pathological diagnosis, which will in turn help to expedite the necessary treatment procedures and bring the required benefits to the patients.

In summary, the study indicates that the tissue floatation bath method, particularly when used with 5% nitric acid, significantly enhances the decalcification process by reducing the time required and improving staining characteristics. However, the choice of decalcifying agent and method should consider the balance between speed, tissue integrity, and staining quality. While 5% nitric acid in the TFB method offers rapid decalcification and superior staining, it also causes discoloration. Conversely, 14% EDTA, despite being gentle on tissue integrity, requires a prolonged decalcification time and leads to more tearing and shrinkage.

Future studies could focus on refining the TFB method and exploring alternative agents or combinations to further optimize the decalcification process. These findings contribute to the ongoing efforts to improve histopathological techniques, ensuring faster, more reliable, and higher-quality diagnostic outcomes in oral pathology.

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