Original

# Comparative Analysis of Effective Decalcification of Tooth with Varying Concentrations of Decalcifying Agents

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## Abstract

Slicing of a given tissue into thin sections is mandatory for microscopic examination. Sectioning of hard tissues like teeth and bone pose great challenge due to their rigidity. Making ground sections of hard tissues is tedious and time consuming. Decalcification is the only reliable method to remove the inorganic portion of these tissues. However the process of decalcification takes a considerable period of time thereby slowing down the process of reporting hard tissue pathologies. Aim: The study aimed at analyzing the rapidity of decalcification of tooth with varying concentrations of formic acid, nitric acid and EDTA. Materials and Methods: 70 freshly extracted human teeth were divided into 10 different groups and each was treated with varying concentrations of decalcification solution until they were completely decalcified. Results and conclusion: Teeth decalcified with nitric acid solution resulted in rapid decalcification compared to other acids. Teeth treated with EDTA showed very minimal removal of inorganic content over a very long period of time. In routine practice 30% nitric acid can be effectively used for rapid decalcification of teeth in the histopathology laboratory however best staining qualities were noted among the teeth decalcified with 10% nitric acid.

## Introduction

Microscopic examination is mandatory to study the morphology and pathology of the given tissue. To obtain satisfactory paraffin sections for pathological examination the hard tissues like teeth and bone should be made flexible by removing the inorganic calcium ions[1]. Decalcification is a process of removal of calcium salts from hard tissues to make them amenable for microtomy and is a prerequisite for microscopic examination of any kind of hard tissue submitted to the histopathology laboratory[2]. Decalcifying agents are the chemicals that are used for the process of decalcification. Decalcification can be carried out by various means including demineralization of the teeth using acids (acid decalcification), ion exchange method, electrolytic method and by using chelating agents. Based on the chemical makeup the acids are further classified into strong inorganic acids and weaker organic acids[3].

Selection of a decalcifying agent depends upon the rate of decalcification, its effect on tissue integrity and staining characteristics[4].

Strong acids like hydrochloric acid and nitric acid produce rapid decalcification but macerate the tissues and decreases nuclear staining when used for a longer periods. Weaker acids like formic acid are less likely to interfere with nuclear staining but much slower in decalcification[3]. EDTA –a chelating agent usually binds to calcium and magnesium ions and removes it from the apatite crystals of the tooth without damaging the organic content. However this process is very slow and might require more than 6 weeks for complete decalcification[5].

The end point of decalcification of the teeth can be assessed by taking x-rays of the teeth, by evaluating the residual calcium in the decalcifying solution and by physically testing the teeth by bending and inserting a pin through the specimen[6]. Resistance to sectioning and effective staining determines the end quality of decalcification. Our study aimed at identifying an ideal decalcifying agent that is both time saving and tissue friendly.

## Materials and Methods

The study sample comprised of 70 freshly extracted permanent human maxillary premolars that were extracted for the purpose of orthodontic treatment after obtaining informed consent. Ethical clearance for the study was obtained from the institutional ethics committee. The samples were divided into 10 different groups each with 7 teeth (Fig 1) and each were treated with varying concentrations of decalcification solution. (Table 1)

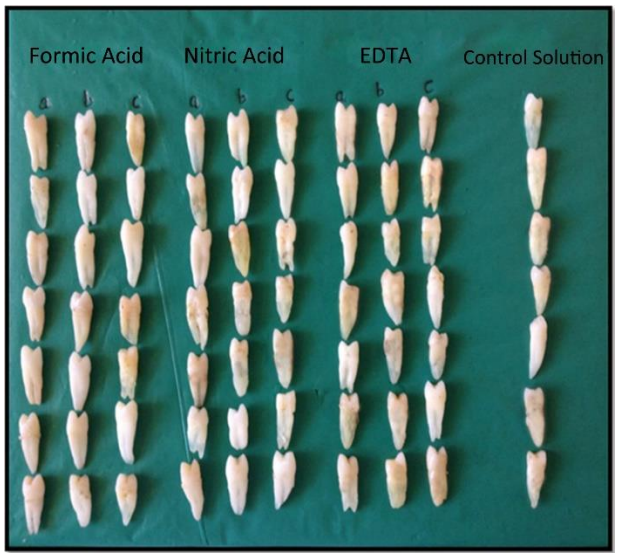


**Figure 1: Photomicrograph showing the teeth samples used for the study**

| S. No | Group | Decalcifying Agent |
|---|---|---|
| 1. | Group I | 10% Formic acid |
| 2. | Group II | 20% Formic acid |
| 3. | Group III | 30% Formic acid |
| 4. | Group IV | 10% Nitric acid |
| 5. | Group V | 20% Nitric acid |
| 6. | Group VI | 30% Nitric acid |
| 7. | Group VII | 10% EDTA |
| 8. | Group VIII | 20% EDTA |
| 9. | Group IX | 30% EDTA |
| 10. | Group X | Commercial decalcification solution (Osteomal) |

**Table 1: Depicting the various study groups and their corresponding decalcifying agents.**

## Decalcification Procedure

The pulp tissue of the extracted teeth were fixed by injecting 10% formalin solution through the apical foramen. A radiograph of each group of the teeth was taken using an occlusal radiograph before the decalcification procedure started.(Fig 2) Each group of teeth was placed in a separate appropriately labelled Plastic container and the concerned decalcification solution was added to each bottle until all the teeth were submerged. The teeth were allowed to decalcify for 8 hours (8.00 am to 4.00 pm). The decalcification solution was discarded at the end of the day; teeth were thoroughly washed with running tap water and were placed in formalin overnight. The teeth were removed from formalin, thoroughly washed and placed in the respective decalcification solution at 8.00am the following day. The procedure was repeated routinely every day until the teeth were completely decalcified. Every evening the teeth were evaluated for decalcification endpoint by using needling (by piercing a needle into the tooth and looking for the resistance) and bending method (by trying to bend the tooth and check its plasticity). The procedure was recorded everyday using a decalcification chart. After decalcification a radiograph was taken for a single tooth of a group using an Intra oral periapical radiograph film. (Fig 3)

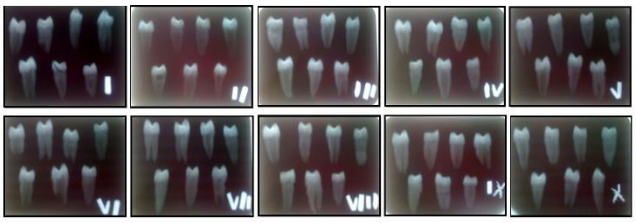

**Figure 2: Photomicrograph depicting the X-ray of the teeth before decalcification.**

**Figure 3: Photomicrograph depicting the X-ray of the teeth after decalcification.**

Upon completion of decalcification all the teeth were routinely processed manually using graded alcohol and xylene, embedded in paraffin wax and 4µm sections were taken and stained with hematoxylin and eosin. The teeth decalcified with EDTA were not processed as the decalcification was not completed within 30 days.

## Results

The teeth that were decalcified with 10% formic acid (Group I) was completely decalcified in 11 days. 20% formic acid solution (Group II) took 10 days and 30% formic acid (Group III) took 9 days respectively. The teeth in Group IV solution (10% nitric acid) underwent complete decalcification in 3 days. Group V solution (20% nitric acid) took 2 days and group VI (30% nitric acid) solution took only one day for complete decalcification.

All the teeth that were decalcified using EDTA (Group VII, VIII & XI) showed very slow decalcification and did not complete decalcification after one month. The control solution which consists of commercial decalcification solution took 5 days for completing decalcification. (Fig - 4)

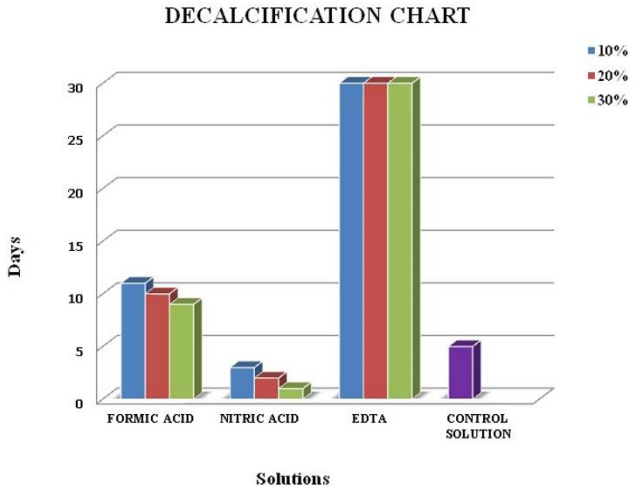


**Figure 4: Bar diagram depicting the duration taken by the different decalcifying solutions for complete decalcification**

Certain amount of yellowish tooth discoloration was noted among the groups which were decalcified with nitric acid solution. Teeth that were treated with formic acid, EDTA and control solution did not show any discoloration.

The staining quality of the tooth section was best in 10% nitric acid compared to other decalcifying agents.(Fig 5) The tooth decalcified with EDTA were not further processed and stained and thus the staining quality of this group was not evaluated. The staining quality of sections of teeth decalcified with osteomol (control solution) were at par with the sections of Teeth decalcified with 10% nitric acid (Fig 6).

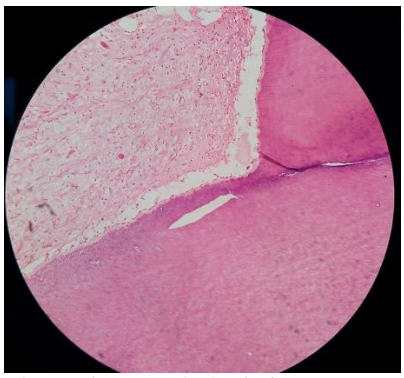

**Figure 5: Photomicrograph depicting Hematoxylin and eosin section of a tooth decalcified with 10% nitric acid.**

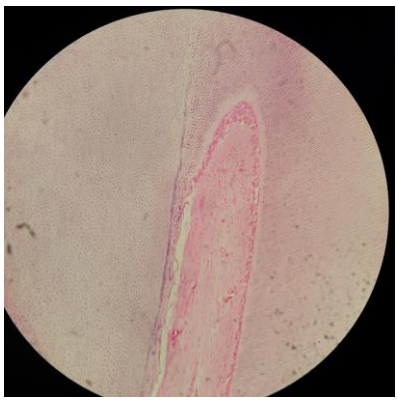

**Figure 6: Photomicrograph depicting Hematoxylin and eosin section of a tooth decalcified with**

## Discussion

The study aimed to evaluate the efficacy and rapidity of various decalcifying agents on human premolar teeth. 30% nitric acid solution produced rapid decalcification within a day, but the staining quality of these teeth was inferior to the staining quality produced by 10% nitric acid which decalcified the teeth in 3 days. Similar study conducted by Prathiba P et al also proved that 10% formal nitric acid produced rapid decalcification in 1.7 days and preneyi's solution took 2.5 days for the same in rat teeth.10% formic acid took 11 days to complete decalcification in our study in contrary to 16.3 days in their study[7]. This observation proves that strong inorganic acids quickly remove the calcium ions but cause severe damage to the organic portions of the teeth.

The teeth decalcified with EDTA showed very minimal changes till 30 days of decalcification. Sanjay.k and Prathiba et al has also proved that EDTA is a very slow decalcifying agent. However with respect to tissue integrity, staining quality and molecular element preservation EDTA produces the best results and can be used for specific techniques like PCR, FISH and ISH[7,8]. Various studies have demonstrated that microwave assisted decalcification of teeth in EDTA solution produced better results compared to decalcification with EDTA alone[9].

## Conclusion

Decalcification is a prerequisite for hard tissue studies. 10% nitric acid is an ideal decalcification agent with respect to both quality and time. EDTA is very slow decalcifying agent and it cannot be considered for urgent specimens, however it is the agent of choice for morphology preservation and molecular studies.

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