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Original Research Article

Comparative and qualitative analysis of various decalcifying agents and their affect on staining and texture of the permanent extracted human teeth

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ABSTRACT

Teeth are among the hardest human tissues, because they are composed of large amounts of inorganic compounds. Teeth are very difficult to prepare for microscopic examination. Chemical Acids and chelating agents traditionally have been used to remove inorganic contents. We compared various decalcifying agents including strong acids, weak acids, chelating agents. Freshly extracted teeth were fixed and decalcified using formic nitric acid, formic acid, nitric acid, EDTA, trichloracetic acid and Perenyi's fluid. Decalcified teeth were evaluated histologically for tissue preservation and staining characteristics.

Aim: The study aimed at analyzing the rapidity and affects the tissue integrity and staining properties of decalcification of tooth with various decalcifying agents.

Materials and Methods: 60 freshly extracted human teeth were divided into 6 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 5% 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 formic acid and EDTA.

1. Introduction

Thin sections of hard tissues are very difficult to obtain by simple methods, which make histological study of tissues such as teeth problematic.[1] Histological examination of hard dental tissues and pulp is imperative for diagnosis of developmental disorders, pulp pathologies, forensic odontology and research.[2] Strong acids, weak acids and chelating agents traditionally have been used to remove calcium ions from hard tissues.[3] Selection of a decalcifying agent depends upon the rate of decalcification, its effect on tissue integrity and staining characteristics Although decalcification by strong acids is rapid, it damages tissue and affects tissue stain ability. Decalcification with weak acids, such as formic acid, preserves tissue details, but is time consuming. Chelating agents, such as EDTA, do not damage tissues or affect stain ability, but they require a long time.[4] Decalcification can be accelerated by applying heat, agitation, vacuum and electric current.[5] Microwave energy and ultrasonic cleaners also have been used to accelerate decalcification.[1] Traditionally, physical, chemical and radiographic methods have been used to determine the end point of decalcification. Apart from these methods, end point determination by precise calculation of residual calcium in the tissues deserves mention. Colorimetric flame photometric and spectrophotometric

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techniques have been used to detect residual calcium in tissue after decalcification.[6]

Our study aimed at identifying an ideal decalcifying agent that is both time saving and tissue friendly.

2. Materials and Method

The study sample comprised of 60 freshly extracted permanent human teeth that were collected between the duration of July 2021- September 2021 from the department of oral and maxillofacial surgery of UCDS Bhairahawa, Nepal. Ethical clearance for the study was obtained from the institutional ethics committee. The samples were divided into 6 different groups each with 10 teeth (Table 1) and each were treated with six different decalcifying agents namely 5% nitric acid, Perenyi's fluid, formalin–nitric acid solution, neutral ethylene diamine tetra acetic acid (EDTA) decalcifying solution, 10% formic acid, and 5% trichloroacetic acid varying concentrations of different decalcification solution. 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.(Figure 1) Each group of teeth was placed in a separate appropriately labeled Plastic container and the concerned decalcification solution was added to each bottle until all the teeth were submerged and decalcifying agents were replaced with freshly prepared solutions every five days. The end point of decalcification of the teeth can be assessed by taking x-rays of the teeth, (Figure 2) by evaluating the residual calcium in the decalcifying solution and by physically testing the teeth by bending and inserting a pin through the specimen. All the teeth were washed under running tap water for 10 min (neutral EDTA decalcified teeth was washed for 2 h) and continued with routine processing, paraffin wax infiltration, and embedding; sectioning and staining with hematoxylin and eosin. The stained sections were observed under the microscope.

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

S.No	Groups (10 extracted teeth)	Decalcifying agents
1	Group I	10% Formic acid
2	Group II	5% Nitric acid
3	Group III	20% EDTA
4	Group IV	10% formic nitric acid
5	Group V	Perenyi's fluid
6	Group VI	5% trichloracetic acid

3. Result

Different decalcifying agents were evaluated and compared on the basis of duration of decalcification of tissue and their effect on the tissue structure.

The teeth that were decalcified with 10% formic acid (Group I) was completely decalcified in 12 days. 5% nitric acid solution (Group II) took 7 days and 20% EDTA (Group III) took 30 days respectively. The teeth in Group IV solution (10% formic nitric acid) underwent complete decalcification in 5 days. Group V solution (Perenyi's fluid) took 55 days and group VI (5% Ttrichloracetic acid) solution took 40 day for complete decalcification. (Table 2) It was observed that 10% formic nitric acid decalcified the teeth fastest followed by 5% nitric acid, 10% formic acid, and 20% EDTA, 5% trichloracetic acid and perenyi's solution being the slowest. (Table 2)

Certain amount of yellowish tooth discoloration was noted among the groups which were decalcified with nitric acid solution and formic nitric acid. EDTA and formic acid did not show any discoloration. Tissue integrity features like pulp organization, arrangement of enamel rods, dentinal tubules are excellent in the group which were decalcified with formic acid and EDTA solutions. Nitric acid and formic nitric acid affect the structural properties of the tissue because of its strong nature. The tissue shows destruction of hard tissue. Neutral EDTA and 5% trichloroacetic acid decalcified teeth stained the best (Figures 3 and 4) and 5% nitric acid and Perenyi's fluid stained the worst (Figures 5 and 6).

Table 2: Shows decalcification period in days

S.N o	Decalcifying agents	Decalcification period in days
1	10% Formic acid	12
2	5% Nitric acid	7
3	20% EDTA	30
4	10% formic nitric acid	5
5	Perenyi's fluid	55
6	5% Trichloracetic acid	40

4. Discussion

Decalcification is the commonly employed technique in most of the histopathology laboratories for the microscopic examination of calcified tissues including teeth and bones.[7] Many decalcifying agents have been developed in search of a balance between rapid decalcification and preservation of morphology. We found that formic nitric acid required the least time for complete decalcification, whereas formalin-EDTA and perenyi's fluid required the most time, which is consistent with the literature (Sanjai et al. 2012, Prasad and Donoghue (2013).[8] Strong acids decalcify most rapidly followed by weak organic acids and chelating acids (Culling 1974).[9] 10% formic nitric acid solution produced rapid decalcification within 5 days, but the staining quality of these teeth was inferior to the staining quality produced by 5% nitric acid which decalcified the teeth in 7 days.

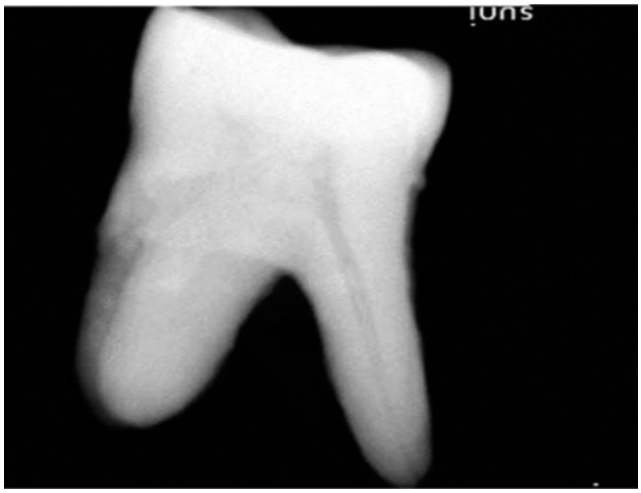

Fig. 1: Radigraph taken before the decalcification

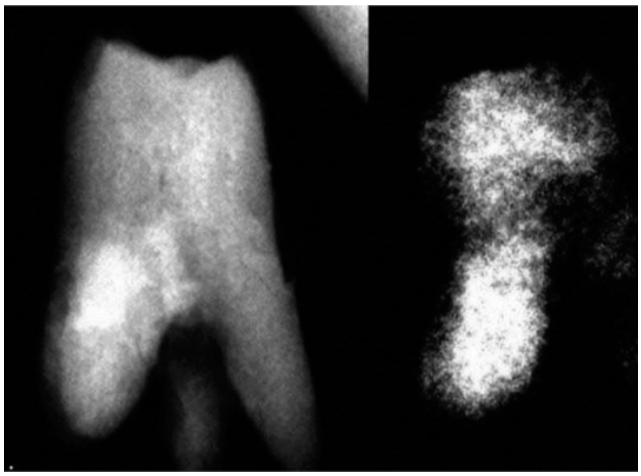

Fig. 2: Radiograph showing end point determination of decalcification of teeth

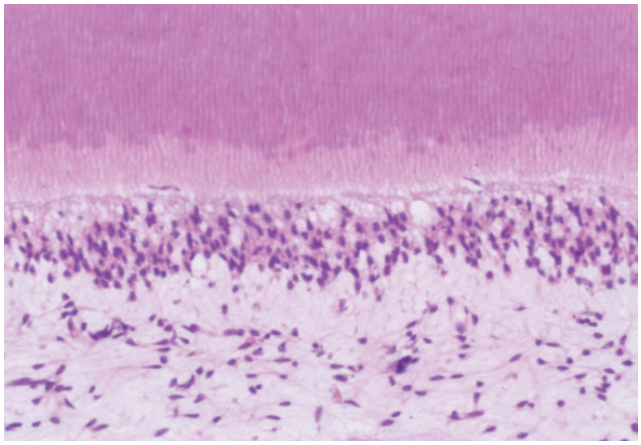

Fig. 3: Tooth decalcified using neutral EDTA decalcifying solution under high

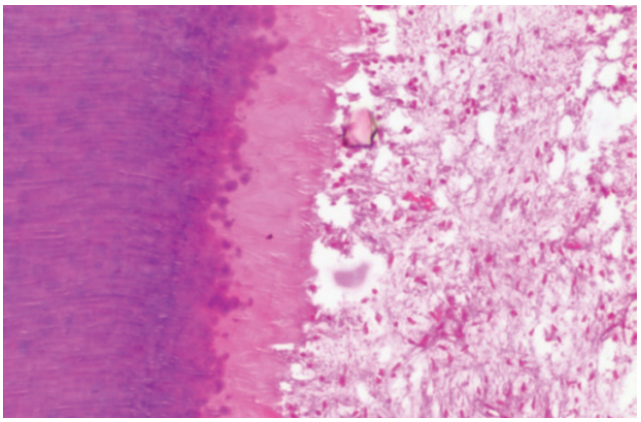

Fig. 4: High-power view of a tooth decalcified in 5% Trichloroacetic acid

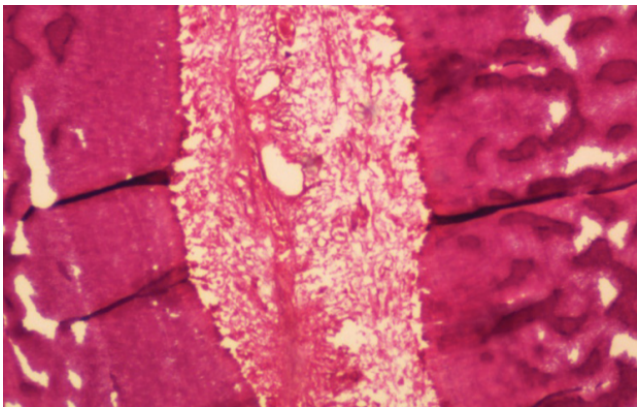

Fig. 5: 5% Nitric acid decalcified tooth in low power

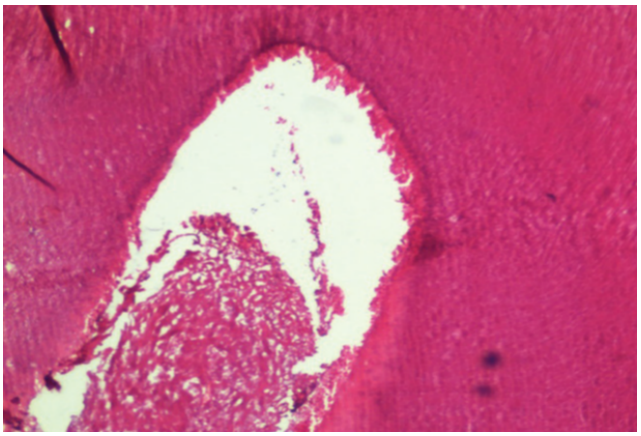

Fig. 6: Tooth decalcified using Perenyi's fluid under low power

Similar study conducted by Prathiba P et al. also proved that 10% formic nitric acid produced rapid decalcification in 3 days and preneyi's solution took 25 days for the same in rat teeth. 10% formic acid took 12 days to complete decalcification in our study in contrary to 16 days in their study.[10] 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 20% 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.[11] When sectioning it was noted that there was crumbling of tissue decalcified in 5% nitric acid and Perenyi's fluid which also contains nitric acid. Also, tissue was indistinct when observed under microscope as also noted by Zappa et al.12 with respect to 7% nitric acid. Teeth decalcified with neutral EDTA responded the best to microtome knife, hence deceiving the physical and radiological methods of testing end point of decalcification with respect to neutral EDTA. In terms of efficacy of agents with respect to soft-tissue integrity and hard- and soft-tissue staining, excellent results were actually obtained with the slowest decalcifying agent, i.e., neutral EDTA.[12] Even 5% trichloroacetic acid also showed good staining characteristics. Soft-tissue attachment and soft-tissue shrinkage, as reported by Zappa et al.[13] suggest that formic nitric acid and nitric acid produce the worst results in contrast to the results obtained from our study, wherein formic acid and EDTA gave good results as it showed minimal soft-tissue shrinkage and minimal loss of tissue. The pulp organization with its extra-cellular matrix and histological zones were clearly distinct and excellent in teeth decalcified with neutral EDTA and 5% trichloroacetic acid.

5. Conclusion

An efficient decalcifying agent should preserve the tissue architecture with a reasonable speed of decalcification for the rapid diagnosis. It balances both tissue integrity and time factor suggesting that it can be used as a stable decalcifying agent for routine histopathological diagnosis. 5% nitric acid is an ideal decalcification agent with respect to both quality and time. EDTA is very slow decalcifying agent and provides good tissue integrity along with excellent staining quality but it cannot be considered for urgent specimens; however it is the agent of choice for morphology preservation and molecular studies. However, further studies are required on a large sample size with consideration of individual factors to evaluate the effect of these agents on dental hard and soft tissues and to find a suitable decalcifying agent which provides reproducible results.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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