SHORT COMMUNICATION

Comparision of 10% Nitric Acid, Edta and 10% Formic Acid for Tooth Decalcification

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INTRODUCTION

Oral tissue received for the histopathology often shows a complex structure involving both hard as well as soft tissues. This structural complexity often makes it difficult to process such tissues for the histopathological diagnosis. The processing of soft tissues involves a bit less complex procedures as they provide lower resistance to the histochemical techniques. Comparing with the soft tissues, hard structures show more resilience towards the histological techniques. So, these tissues require more complex and sensitive technique to process them for the histological diagnostic procedures.

The pulpal soft tissue resides within a closed chamber surrounded by hard tissue (i.e. dentin) on all sides except the apical foramina. So, it is difficult to visualise the histological of structure of the pulp without cutting through the hard tissue which may have deleterious effects on the soft pulpal tissue. The method which is employed for cases like this is the process known as decalcification. Decalcification involves a complete removal of calcium

Decalcification of hard tissues has remained an important part in visualizing the histology of the section. For the process of decalcification various decalcifying agents have been used in the past but very limited studies can be found in the literature about the comparison of various decalcifying agents. Aim: Here we present a study comparing three different decalcifying agents (10% Nitric acid, EDTA, 10 % Formic acid). Methodology: 30 freshly extracted premolars were decalcified using three different decalcifying agents. The end point of decalcification was tested by chemical methods. The decalcified sections were routinely processed and staining was done using Hematoxylin and Eosin. Result: Sections decalcified with EDTA gave better results as compared to the other two groups. Conclusion: EDTA is a better option for decalcification of tooth but it has a slow rate of action. So, for the cases requiring urgent results nitric acid can be used. Key words: Decalcifying agents, EDTA, Decalcification, Nitric acid, Formic Acid.

> salt from mineralized tissues like teeth, bone and other calcified tissues. The property of physical hardness is a unique characteristic which makes it necessary to "soften" them by removing the mineralized component.¹ A number of decalcifying agents have been used from the past for the process of decalcification such as acids, chelating agents etc. Here we present a study to check the effective ness of few decalcifying agents. the aim to compare 10% nitric acid. EDTA and 10% formic acid for decalcification of tooth.



METHODOLOGY

The study was done on 30 non carious, non attrited and freshly extracted human premolar teeth. The teeth were extracted for orthodontic purposes and were obtained from patients of the age group 20-30 yrs. The teeth after extraction were immediately transferred to a tight container containing 10% fromalin for fixation and preservation upto 24 hrs after which they were subjected to decalcification. Three decalcifying agents namely 10% nitric acid, EDTA and 10% formic acid were used. The tooth samples were suspended using a thread in 3 different coplin jars containing 100 ml of decalcifying agent. The starting time for decalcification was noted and the temperature and were recorded on regular basis. The solutions of neutral 10% formic acid and EDTA were replaced with fresh solutions after every five days and the decalcifying agents were subjected to repeated agitation. The end point of decalcification for both acids and EDTA was estimated using chemical test.^[1]

The teeth were then washed under running tap water for 15 min and for neutral EDTA decalcified teeth were washed for 2 hrs. The teeth were then subjected for routine processing, paraffin wax infiltration and embedding; sectioning and then staining with hematoxylin and eosin.^[2]

Criteria for observation^[2]

Speed of decalcification: 1-5 [slowest to fastest].

The stained sections were graded on the microscopic examination on the basis of following criteria:

1 to 4 [1-poor, 2-fair, 3-good and 4-excellent]

- 1. Ease of sectioning.
- 2. Hard-tissue staining.
- 3. Soft-tissue staining both cytoplasmic and nuclear staining.
- 4. Soft-tissue attachment.
- 5. Soft-tissue shrinkage.
- 6. Pulpal organization.

RESULTS

Comparing the speed of action of the above-mentioned decalcifying agents, 10% nitric acid proved to be the fastest in its action followed by 10% formic acid. EDTA had a very slow rate of action and stood last in the group (Graph 1). While comparing the other parameters such as ease of sectioning, soft tissue attachments, staining properties etc. EDTA produced the best results as compared with the other two decalcifying agents followed



Graph I: Comparison of Speed of action of Various decalcifying agents.

Table 1: Comparison between the decalcifying agents on the basis of various parameters.

Parameters	10% Nitric Acid	EDTA	10% Formic Acid
Ease of sectioning.	1	2	4
Hard-tissue staining.	2	3	4
Soft-tissue staining: both cytoplasmic and nuclear staining.	1	2	3
Soft-tissue attachment	1	1	3
Soft-tissue shrinkage.	1	2	3
Pulpal organization.	1	2	4
TOTAL SCORE (Maximum score: 24)	7	12	21

by 10 % formic acid and then 10% Nitric Acid last in the group (Table 1).

DISCUSSION

Biopsies obtained from the head and neck region often show a complexity in their structure as they include both soft and hard tissues.^[2] As compared with soft tissue the biopsy samples including hard tissue such as bone and teeth often need a more complex method such as hard-tissue grinding and decalcification^[3] to make them available for the histopathological diagnosis. Pulp is a soft tissue cased within the hard structure i.e. dentin. Procedures like hard tissue grinding can have adverse effect on the pulp and may even result in the loss of the tissue structure. So, the best method to visualize pulpal structure histologically is decalcification. Decalcification also termed as demineralization is a process carried out routinely in most of the laboratories by decalcifying agent's agents.^[1] These may be acids, chelating agents etc. The acidic decalcifying agents act through a diffusion system forming soluble calcium salts, but in case of a chelating agent like EDTA, decalcification acts by binding calcium ions that form stable EDTA-Ca reactions.^[4] There are various factors such as solution concentration, temperature, exposure time and penetration rate^[5] which influence the action of a decalcifying agent. Control over these parameters is necessary for the proper action of the decalcifying agent as well as to achieve a good histological section of the tissue.

Use of a decalcifying agent in an inappropriate manner can often lead to tissue damage, usually characterized by the loss of cytoplasmic and nucleic staining.^[6] So it is necessary to determine the decalcification endpoint specific to commonly used agents. A number of test including physical (physical testing by probing or bending to detect hardness, mechanical testing by needling), chemical method (chemical detection of calcium ions in the decalcification solution, bubble tests) and radiographic detection of calcium in the specimen)^[7] are being used to test the end point of decalcification. However, disadvantages such as generation of artefacts, destruction of cellular detail and false-positive readings can be encountered while using physical and chemical tests.^[5]

In the present study we compared three decalcifying agents namely 10% Nitric acid, EDTA and 10% Formic acid. The efficiency of these agents was compared on the following parameters of Speed of action, Ease of sectioning, Hard-tissue staining, Soft-tissue staining – both cytoplasmic and nuclear staining, Soft-tissue attachment, Soft-tissue shrinkage, Pulpal organization. In our study it was found that the speed of action of 10% nitric acid was fastest followed by 10% formic acid. The process of decalcification with EDTA was the slowest among the three. Our results were in accordance with Zappa *et al.*^[8] Sanjai *et al.*^[2] and Mattuella LG *et al.*^[9] who also found in their studies

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that the speed of decalcification was slowest by using EDTA.^[10]

Comparing the other parameters EDTA gave the best results in every aspect as compared with the other two group's i.e. 10% nitric acid and 10% formic acid. The soft tissue integrity was almost lost with Nitric acid (Figure 1) and Formic acid (Figure 2) and was well preserved with EDTA (Figure 3). Our results were comparable to the studies done by Zappa *et al.*^[8] who showed that HNO₃ and FA were showing worst results after decalcification, for both hard and soft tissue components of tooth as compared to EDTA and other agents used in their study. Also, it has been found in the studies done by Sanjai K *et al.*^[2] and Singh S, Sarkar K^[11] that



Figure 1: Tissue section decalcified with 10% Nitric acid showing loss of soft tissue.



Figure 2: Section decalcified with 10% Formic Acid showing Loss of attachment of the soft tissue.



Figure 3: Section decalcified with EDTA showing well preserved hard and soft tissue.

overall results were best shown by EDTA.

Neutral EDTA gave superior results and this may be due to capturing of metallic ions like calcium that binds with the chelating agent. It means that calcium ions from the external layer of the apatite crystals will be removed and when all the calcium are removed from the external layer, then ions from deeper layer will replace them. In this way, the crystal size decreases gradually, producing an excellent preservation of tissue components.^[11]

CONCLUSION

According to the results obtained we may conclude that both acids as well as chelating agent have their own merits and limitations. In our study EDTA showed the best results as compared to the other groups in every aspect except time. As the process of decalcification is slow with EDTA so for cases of urgency Nitric acid can be employed for hard tissue decalcification. In future, further studies can be performed with increased tooth samples as well as increased number of decalcifying agents that might get us close to a decalcifying agent that is fast and has lower deleterious effects on the tissue structure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

None.

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