ORIGINAL RESEARCH PAPER

Oral Pathology

DECALCIFICATION OF TEETH – AN UPDATE

KEY WORDS:

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ABSTRACT

Decalcification is a critical and skilled technique used in oral histology to prepare tissue samples for microscopic analysis. It is a routinely employed technique in oral histology and pathology. It plays a highly amenable role in diagnosing hard tissue pathology by making it easy for processing and sectioning. This process involves the removal of mineral deposits from hard tissues such as teeth and bones, allowing for clearer visualization of the tissue's structure and cellular components. Various staining techniques and recent advances are blooming in this field of histology. Decalcification is often necessary for the diagnosis of various oral diseases and abnormalities, including dental caries and periodontal disease. Understanding the principles and methods of decalcification is essential for successful tissue preparation and accurate diagnosis in oral histology. This article provides a brief overview of decalcification in oral histology, emphasizing its importance and application in dental research and clinical practice.

INTRODUCTION

Decalcification is the process by which the inorganic calcium is removed from the hard tissue before processing the specimen. This can be done by using either acids which forms soluble calcium salts or by using chelating agents that will bind to calcium ions and aids in decalcification or by electrolytic method. Bone decalcifications are widely and commonly used in histopathology for the diagnosis of various pathological conditions, similar to bone decalcification, tooth decalcification are also widely used nowadays for diagnosis of certain developmental tooth anomalies such as dentin dysplasia. And sometimes these techniques are used by researches in identification of molecular pathogenesis, pulpal response to the restorative material and pulpal calcifications. There are several studies conducted to compare the efficacy of different decalcifying agents. In some pathologies involving the oral cavity, were the bone or tooth is attached with the lesion and those conditions hard tissues along with soft tissues cannot be processed at the same time. These small foci of calcified mass will interrupt during sectioning, causes damage to the knife or disruption of surrounding tissue. This review describes the importance of tooth decalcification, various techniques employed, end-point decalcification tests and their applications in dentistry. (1,2)

FACTORS THAT DETERMINE THE CHOICE OF DECALCIFYING AGENTS

- Urgency of the case
- Degree of mineralization
- Extent of the investigation
- Staining technique required

DECALCIFYING AGENTS

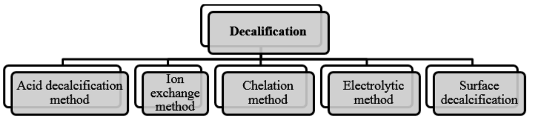
In previous day's acids and chelating agents was only used, later Gray in 1954 listed over 50 different mixtures of decalcifying agents. Recently there are many commercially available decalcifying agents were developed, which also follows the same formula which was used several years ago. Those mixture consists of addition of fixatives such as formalin and buffer salts to compensate the effects caused by acids. For routine work, nowadays the laboratory uses simple decalcifier which provides better results, which also depends on the type and size of specimen used. (3)

CRITERIA FOR GOOD DECALCIFYING AGENT

Complete removal of calcium, Minimum damage to cells and tissue, Non-impairment of subsequent staining and Speed

CLASSIFICATION

Decalcification of tooth can be classifies based on methods employed and quality of the decalcification and good staining flexibility. (Cullings text book of lab techniques)



RECENT ADVANCES

There have been recent advances in decalcification and staining techniques that have improved the quality and efficiency of oral histological examination.

- EDTA decalcification
- Ultrasonic Energization in decalcification
- Decalcification by perfusion method
- Microwave oven decalcification
- Toluidine blue staining
- Digital pathology (1,4,5)

EDTA decalcification

Ethylene di-amine tetra acetic acid (EDTA) has shown to be an effective decalcifying agent for oral tissues. It is a chelating agent that binds to calcium ions and removes them from tissues without damaging the tissue structure. It has been shown to produce better tissue preservation and retention of antigenicity, compared to traditional methods using strong acids - Rebecca et al. 2021 (5)

ULTRASONIC ENERGIZATION:

Ultrasonic Energization involves using high-frequency sound waves to create cavitations bubbles in the solution used for decalcification. These bubbles implode, creating localized high-pressure and high-temperature zones that help break down the mineral deposits in the tissue. The use of ultrasonic Energization in decalcification can significantly reduce the time required for the process and improve the quality of the decalcified tissue. It can also reduce the risk of tissue damage compared to other decalcification methods that use harsh chemicals or prolonged exposure to acids. However, it's important to note that ultrasonic Energization can also cause damage to the tissue if used at too high of an intensity or for too long of a duration. Therefore, it's crucial to optimize the parameters used during ultrasonic Energization to ensure the best results and minimize tissue damage. (6)

DECALCIFICATION BY PERFUSION METHOD:

The use of a perfusion apparatus allows the decalcification solution to circulate through the tissue, ensuring uniform decalcification throughout the tissue. The perfusion apparatus consists of a container filled with the decalcification solution and a pump that circulates the solution through the tissue. The tissue is placed in a chamber and connected to the pump using a catheter. The decalcification solution is then circulated through the tissue at a controlled rate, allowing the solution to penetrate the tissue and remove the mineral deposits. The decalcification solution used in the perfusion method usually contains a chelating agent, such as ethylene di-amine tetra acetic acid (EDTA), which binds to the calcium ions and removes them from the tissue. The perfusion method is preferred over other decalcification methods as it allows for uniform and controlled decalcification without causing damage to the tissue. The perfusion method is commonly used in research settings, particularly for the preparation of tissue samples for histological analysis. The decalcified tissue can then be processed, sectioned, and stained for microscopic examination. This technique is also used in the preparation of bone grafts for transplantation, as the decalcified bone can be reshaped or reformed to suit the patient's needs. (7)

MICROWAVE DECALCIFICATION:

Among various recent advances in decalcification microwave decalcification has gained popularity. One of the modifications of microwave decalcification with auxiliary board works perfectly, but it should be accompanied with a safety circuit which increases the reliability and helps in continuous processing. Patchy staining artefact is very much and significantly minimal while using microwave decalcification technique. It speeds up the action of the chelating agents. It has used the ability of the microcontroller in commercial microwave oven and had converted it into a lab instrument. Furthermore it is quite budget friendly when compared to other lab expensive counterparts. It takes relatively lesser time when compared to manual methods of complete decalcification. With all three decalcifying agents (nitric acid, formic acid, EDTA) microwave decalcification method shows better preservation of tissue when compared to routine method. (8)

TOLUIDINE BLUE STAINING:

During the decalcification process, the hard tissue undergoes significant changes in its chemical and physical properties. The calcium ions are removed from the tissue, resulting in a significant loss of tissue integrity and the formation of large gaps in the tissue structure. Toluidine blue staining can be used to visualize these gaps, which appear as empty spaces in the stained tissue. To perform toluidine blue staining in decalcified tissues, the tissue sections are immersed in a solution of toluidine blue dye for a few minutes. The dye binds to the acidic components in the tissue, staining them blue. The tissue sections are then rinsed in water, dehydrated, and mounted for microscopic examination - Watanabe, T., Kato, H., & Kimura, Y. (2018) (2,6,7)

DIGITAL PATHOLOGY:

Digital pathology has significant applications in the decalcification process, as it allows for high-resolution imaging and analysis of decalcified tissue samples. The use of digital pathology in decalcification enables researchers and pathologists to visualize and analyze tissue samples in a non-destructive manner. This technology allows for the generation of high-resolution images of the tissue samples, which can be viewed and analyzed remotely by multiple users simultaneously - Della Mea, V. (2018) (9)

APPLICATIONS: (Table 1) (6, 7, 10)

Examination of Hard Tissue Structure:	The process involves removing the mineral component of the tissue, which makes it easier to cut into thin sections and stain the tissue for analysis. This technique is particularly useful in the study of dental tissues such as dentin, pulp and bone structures.
Identification of Pathological Changes:	Decalcification is often used to identify pathological changes in hard tissues. I.e. Dental caries, in which decalcification can be used to visualize the extent of the lesion and to identify the bacteria involved in the process. Similarly, in bone diseases such as osteoporosis, decalcification can be used to examine changes in bone structure and density.
Research in Oral Histology:	It is used to study the internal structure of hard tissues and to examine changes in response to disease or treatment planning. This technique is particularly useful in the study of dental materials and the development of new treatments for dental diseases.
Diagnostic Applications:	It can be used to prepare biopsy samples for examination to determine the presence of tumours or other pathological conditions.
Forensic Investigations:	It is commonly used technique and valuable method for identification of teeth, and to determine the age, sex, and other characteristics of the individual.

FUTURE DIRECTIONS IN DECALCIFICATION TECHNIQUES: (Table 2)

Future directions in decalcification techniques are aimed at improving the quality of histological samples while minimizing tissue damage and reducing processing time. Here are some potential areas of focus for future research: (7, 9, 10, 11)

Development of novel decalcification agents	There is ongoing research to develop new decalcification agents that are more effective and less damaging to tissue than current agents. One area of focus is the development of decalcification agents that are less acidic and less harmful to tissue.
Alternative decalcification methods	There is growing interest in alternative methods for decalcifying tissues, such as microwave-assisted decalcification, enzymatic digestion, and mechanical decalcification. These methods have the potential to reduce processing time and produce higher quality histological samples.
Automated decalcification	Automation of decalcification processes has the potential to improve productivity and cut down on processing times while maintaining high-quality histological samples. Robotic systems, which can carry out decalcification processes with more accuracy and precision than manual methods, can be used to accomplish this.
Digital pathology	The use of digital pathology can enhance the analysis of histological samples and can be applied to decalcified samples. This technology can improve the accuracy and speed of image analysis, allowing for more detailed and comprehensive analyses of histological samples.

Integration of decalcification with other processing techniques	There is growing interest in integrating decalcification with other processing techniques, such as Immunohistochemistry and molecular analysis. This can provide a more comprehensive analysis of tissue samples and facilitate the identification of biomarkers and other molecular targets.

CONCLUSION

Decalcification of bone is widely used method in histopathology, whereas decalcification of teeth is also widely done in the oral histopathology labs. Decalcification is a process used in oral histology to remove the mineral content from teeth or other hard tissues for microscopic examination. This procedure is essential in understanding the structure and function of teeth and diagnosing various dental diseases. In oral histology, decalcification is used to examine various dental diseases such as dental caries and periodontal disease. It also helps in the evaluation of dental materials and the study of the structure and function of teeth.

Hence, the decalcification process is an essential procedure in oral histology that allows for the microscopic examination of teeth and other hard tissues. It provides valuable information to dental professionals to help diagnose and treat dental diseases and maintain good oral health.

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