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Ground Sectioning- An Overview

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ABSTRACT

The histological evolution of hard tissues, such as bone is a complex process that requires careful consideration of tissue preparation and sectioning techniques. The integrity of both soft and hard tissues must be preserved to enable accurate histochemical evaluation, making fixation a crucial initial step. Decalcification followed by paraffin embedding and freezing or plastic embedding are common approaches used to prepare hard tissues for histological analysis. this study compares the preparation method of ground, decalcification and resin embedded sections of human bone to determine the most widely accepted and efficient approaches for studying bone histology. The findings of this review will inform the development of standardized protocols for the histological evolution of hard tissues, ultimately enhancing our understanding of cellular and subcellular architecture and activities in these complex tissues. This review examines the challenges of working with bone containing specimens, including the mixing of bone and soft tissue and the need for uniform softening or hardening of the tissue to facilitate sectioning.

KEYWORDS: Ground sectioning, hard tissue, oral histology

INTRODUCTION

Teeth are highly specialized structures that serve critical roles in the mechanical breakdown of food, speech, and maintaining the facial appearance. The hard tissues of teeth, which are primarily composed of enamel, dentin, and cementum, are among the hardest biological substances in the human body. These tissues are carefully structured to provide strength, durability, and resilience, necessary for the rigorous forces involved in chewing and biting. A standardised thin section approach is necessary for the histological evaluation of hard tissues using light microscopy techniques [1]. Because it is challenging to preserve the integrity of the tissues (both soft and hard tissue) in such a situation, histochemistry evaluation of living tissue cannot be performed. Fixing the tissue is therefore a crucial first step in all histological processing and tissue sectioning [2]. Understanding cellular and subcellular architecture and activities requires the reservation of hard tissues. Histological work with bone and bone-containing specimens is intrinsically challenging [3]. Because bone and soft tissue (bone, fat, or neoplastic tissue) are intimately mixed, cutting sections of bone marrow biopsy specimens, bone cancer samples, or biopsies of metastases to the bone can provide challenges. The tissue can be made uniformly soft by decalcification followed by paraffin embedding, or it can be made uniformly hard by freezing fresh material or by embedding in plastic in order to cut sufficient unbroken portions [4]. Following the hard tissue's processing and sectioning, the sample's organic and inorganic content serves as

the basis for the histological analysis [5]. Both the body's teeth and bones are considered hard tissue. In order to determine which approach is more widely accepted and makes studying bone histology easier, this review is based on the histological preparation of ground, decalcified, and resin-embedded sections of human teeth.

FIXATION

When fresh tissue is kept at room temperature, it becomes liquified and smells bad. This is because putrefaction and autolysis are causing alterations in the tissues.

The goal of the fixation process is to preserve the tissue components so that thin, stained sections can be prepared [2].

By giving the treated materials mechanical strength and stability, it maintains the tissue's structure. Fixatives change the tissues to improve the cell's integrity.

The first and most important step in a multi-phase procedure to get a biological material sample ready for microscopic inspection is fixation.

There are three types of fixation: perfusion, immersion, and heat fixation.

Both perfusion and immersion involve chemical fixatives. The typical chemical fixative for paraffin embedded sectioning is neutral buffered formalin (NBF) [6].

This is the same as 4% paraformaldehyde in a buffered solution plus methanol, a preservative that stops formaldehyde from turning into formic acid.

The standard fixative for all sectioning and preservation techniques is formalin.

ADVANTAGES:

1.Detailed Internal Examination:

Ground sectioning provides a clear view of the tooth's interior tissues, including enamel, dentin, pulp, and root canals. This generates high-resolution photographs of the tooth's microstructure, which is essential for studying tooth decay, developmental anomalies, and the impacts of restorative materials.[7]

2.Accurate Measurement of Tooth Structures:

Ground sectioning allows for precise measures of enamel and dentin thickness, pulp chamber size, and root canal morphology that other imaging modalities, such dental X-rays, may not give.[8]

3.Enhanced Visualization of Tooth Decay:

Improved imaging of Tooth Decay Ground sectioning allows for microscopic imaging of enamel and dentin lesions, aiding in early detection of tooth decay or mineral loss. It can detect carious lesions and cracks that are not usually visible with typical radiography approaches.[9]

4.Study of Tooth Wear and Erosion:

Study of Tooth Wear and Erosion Ground sectioning is a valuable tool for investigating tooth wear patterns, enamel loss, and the impact of abrasion and erosion over time. Researchers can better understand how teeth wear by analysing small, cross-sectional slices of enamel.[10]

5.**Reveals Internal Changes Due to Dental Treatment:**

Ground sectioning reveals internal changes caused by dental treatment. This enables for the study of how restorative materials (e.g., fillings, crowns, sealants) interact with the tooth's internal structure over time. Ground sections can be used to analyse the impact of dental procedures such as bonding and pulp tissue reaction to dental materials.[11]

6.Cost-Effective for Small-Scale Studies:

Cost-effective for small-scale studies. Advantage: Ground sectioning is a less expensive imaging technique than scanning electron microscopy (SEM) or X-ray microtomography. Ground sectioning can be a useful and cost-effective tool for small-scale research or education.[12]

7. Good for Histopathological Studies

Ground sectioning provides the ability to perform histopathological analyses of dental tissues. It is particularly useful for identifying and studying the microscopic characteristics of teeth in diseases such as pulpitis, periodontitis, and other dental pathologies.[13]

8. Facilitates Longitudinal Studies

Ground sectioning can be used in longitudinal studies to track changes in tooth structure over time, particularly when studying the effects of various external factors such as diet, aging, or mechanical stress. It provides a physical record of tooth changes that can be revisited and reanalysed at different stages.[14]

CLINICAL APPLICATION:

1.Assessment of Dental Caries (Tooth Decay):

Ground sectioning is used to study the evolution of dental caries (decay) at the microscopic level. It reveals the degree of enamel and dentin demineralization, enabling early detection and accurate assessment of carious lesions that may not be seen in traditional radiographs.[9]

Clinical Relevance: Early detection of caries is crucial for avoiding further deterioration and guiding conservative restorative therapy.[9]

2.Evaluation of Restorative Materials:

Ground sectioning can be performed to evaluate the interaction of restorative materials (such dental composite resins and glass ionomer cements) with tooth structure. It aids comprehension of how these materials adhere to enamel and dentin, as well as their long-term durability and wear resistance. [11]

Clinical Relevance: This allows doctors to select the most appropriate materials based on their interaction with the tooth structure, resulting in better patient results in terms of longevity and aesthetics.[11]

3.Root Canal Analysis:

Ground sectioning is performed in endodontics (root canal therapy) to examine the root canal system, including its morphology and variations. It also assesses the sealing ability of endodontic materials and the presence of any gaps or cavities in the root filling.[15]

Clinical Relevance: Ground sectioning offers vital information on the effectiveness of root canal treatments and the quality of root filling, which is useful in predicting the success or failure of endodontic therapy.[15]

4.Study of Tooth Wear and Erosion:

Ground sectioning is commonly used to analyse dental wear due by bruxism (teeth grinding), abrasion, and erosion (acidic food or beverages). The approach allows clinicians to see the loss of enamel and dentin at a microscopic level, allowing them to create appropriate preventive and restorative treatments.[10]

Clinical Relevance: Understanding the mechanisms and amount of tooth wear aids in the diagnosis and management of disorders such as dental erosion and abrasion, as well as the development of preventive interventions such as remineralization or restorative treatments.[10]

5.Assessment of Enamel Thickness and Dentin Structure:

Ground sectioning is used to determine the thickness of enamel and dentin in different areas of the tooth. This is critical for assessing a tooth's susceptibility to caries, response to restorative therapies, and overall health.[8]

Clinical Relevance: Understanding enamel and dentin thickness allows you to make more informed decisions about restorative therapies such crown preparations, root canal procedures, and minimally invasive treatments.[8]

METHODOLOGY FOR GROUND SECTION

Certain structures get obscured as a result of bone and tooth decalcification. A saw, a forceps lab lathe, a fine and coarse abrasive lathe wheel, water directed onto a rotating wheel, a wooden block, adhesive tape, a brush, ether, mounting medium, microscopic slides and cover glass, a scalpel, and reagents such as 50% alcohol, xylene, and DPX are among the tools used to grind a section of hard tissue [16,17].

Using a coarse abrasive lathe wheel first, then a fine abrasive lathe wheel, the cut surface of the hard tissue (teeth) is ground. A fine abrasive lathe wheel was used to smooth the surface after the tissue was crushed down to a thickness of roughly 0.5 mm. After the ground section was finished, it was soaked in ether for a few minutes and then dried for a few more.

In order to prevent cracking, the ground area of the tissue should not be allowed to dry for an extended period of time [7]. While grinding, a continuous mist of water and Paris powder must be applied to the grinding surface. Paris powder keeps teeth from grinding unevenly, and water helps cool teeth that heat up from grinding friction [18].

On the flint paper, which was maintained on a level, stable surface, was the transverse bone segment. Grinding was halted once the segment reached a transparency of 25–30 microns, and it was then rinsed under running tap water. The transparent portion was cut with a scalpel and forceps after the piece was examined under a microscope.

A contemporary trimmer with water for cooling was used to trim the hard tissue (teeth) that was embedded in the plaster slab. After being crushed to a thickness of about 4-5 mm, the tooth was moved to a bench and ground for another 2-3 mm. After that, the coarse abrasive is swapped out for fine abrasive and cut to 1 mm.

For removing imperfections and polishing the area on Arkansas stone was then polished with a pumice and water slurry to make it paper-thin and smooth [19]. After that, a cover slip and DPX solution are used to place it on the slide.

A handsaw was used to cut the human bone's femur in a diaphyses section. Four distinct approaches were employed. Wet SiC13 was used to grind the transversal sections in Method 1, followed by carborundum in Method 2, carborundum after the cross sections were immersed in methyl methacrylate solution for 24 hours

in Method 3, and carborundum once more following the application of a few drops of cyanoacrylate glue to the bone's periosteal surface and the surface created by the initial cut in Method 4.

The sections were covered with cover glass after being ground and adhered on regular glass microscopic slides.

The parts were covered with glass and then gradually heated (carbonised). Sections were ground, then placed in distilled water with a few drops of detergent, allowed to air dry, sealed in hot Canada balsam, and covered with cover glasses dipped in m-xylene.

Following grinding, the sections were cleaned, allowed to air dry, then submerged in 95% ethanol, let to air dry one more, and then covered with methyl methacrylate solution.

METHODOLOGY FOR DECALCIFIED SECTION

In order to create high-quality paraffin sections that retain all of the crucial microscopic elements, decalcification is the process of removing minerals from bone or other calcified tissue.

After the specimen has been completely fixed and before it is processed to paraffin, decalcification is done. Strong mineral acids, weaker organic acids, and cheating agents are the three primary categories of decalcifying agents [2].

These days, tissues can also be decalcified using the electrical approach [3]. Decalcification comes after the specimen has been fixed. The specimen is suspended in roughly 400 millilitres of 5% nitric acid in the test tube.

For eight to ten days, the acid should be replaced every day, and its full decalcification is checked. Decalcification's ultimate point is crucial, although it might be challenging to evaluate.

To get rid of the acid, the decalcified specimen needs to be cleaned for a full day. The specimen should be dehydrated for 24 to 48 hours using an increasing proportion of alcohol after washing.

Parlodion should then be used to penetrate the specimen. Pure nitrocellulose dissolved in ether alcohol is called parlodion. After being moved to 2% parlodion, the specimen is thereafter moved to an increasing proportion of parlodion.

The size of the substance and the quantity of bone and dental material present determine how long it takes to infiltrate. Following parlodion infiltration, additional parlodion is added until the thickness of the parlodion above the specimen is approximately 13 mm. After that, the specimen is positioned in the appropriate cutting plane.

After that, a lid was placed over the disc to allow the ether alcohol to slowly evaporate. Parlodion solidifies as it evaporates. It takes at least two to three weeks to harden. Following this, the block was submerged in chloroform and then removed using 70% alcohol. Additionally, the specimen is dyed after being sectioned using a sliding microtome.

This embedding method can be modified by substituting acid celloidin for parlodion. This keeps the tooth enamel's biological matrix intact while it decalcifies [7]. 50 cc of HCl acid at varying concentrations of 0.4, 0.2, 0.1, 0.05, and 0.025 N was given to one group of bones.

One gramme of the other group is subjected to ethylenediaminetetraacetic acid (EDTA), a tetrasodium salt. The remaining 5 grammes of bone were then treated to phosphate-buffered disodium salt at a pH of 7.4 [4].

METHODOLOGY FOR RESIN EMBEDDED SECTION

Hydrophilic resins minimise the denaturation of proteins. Glutaraldehyde is used as a fixative for resin embedding (4% in 0.1 M phosphate buffer).

Fixation is followed by alcohol-based dehydration, after which resin is infused to replace the alcohol. Before sectioning, extra resin is cut off.

Diamond or glass knives work best for sectioning. Epoxy resin prefers dry sectioning, whereas methacrylate prefers wet sectioning [5].

Another option is composite resin, which has to cure for 60 seconds [1]. Following a sequence of graded ethanol dehydration, the samples were infiltrated three times for 40 minutes at the proper temperature using lowicryl and bioacryl.

The mixture was securely sealed and agitated gently until the catalyst had dissolved after 1.3 g of benzoyl peroxide was introduced as a catalyst. Samples infiltrated with both bioacryl and lowicryl were polymerised in Ep-pendorf tubes using UV light for 72 hours at 4°C.

Following ultramicrotome sectioning of the embedded material, different histological stainings were applied [6].

As an invading solution, 9g of JB-4 resin component A, 1g of methyl methacrylate monomer, and 45mg of dried benzoyl peroxide were utilised. 10g of pre-chilled infiltrating solution and JB-4

HISTOLOGY OF GROUND SECTION, DECALCIFIED SECTION AND RESIN EMBEDDED SECTION

Under low power magnification, the ulna's ground section has osteons with concentric lamellae.

Each osteon has a Haversian canal in the middle, lacunae between the osteocyte-containing concentric lamellae, Volkmann's canals that connect Haversian canals in a rung-like ladder, and interstitial lamellae between Haversian systems [8].

A small ground slice of the tooth can be used to study the cementum, enamel, and dentin [10].

Beautiful sections can be seen under a microscope at a magnification of 200 on the ground section created by carborundum once more after a few drops of cyanoacrylate glue have been applied to the bone's periosteal surface and the surface created by the initial cut.

Differentiated secondary osteons and some of them connected with Voklmanns cannals are seen. lot of interstitial lamellae in between the particular osteons were also seen in the sectional microscopic view. But the outer circumferential lamellae were not identified [1].

Decalcification is a procedure in which inorganic content of the soft or hard tissue is removed after fixation. lacunae containing osteocytes shows up as dark spots.

Canals carrying capillaries (cap) and nerves are seen as larger open areas in bone. Since inorganic components are removed from the hard tissue the microscopic view is not as clear as ground section.

Since the ground section no longer contains organic material, the inorganic structures are plainly visible there, but the decalcified section displays the organic structures.

Improved morphological features and minimal bone marrow cell shrinking are seen in the resin-embedded slice. Each and every periosteal and endosteal bone exhibits osteoclast. There was evidence of leukocytes and osteoblasts [8].

LIMITATIONS:

1. Destructive Nature of the Process

Ground sectioning is an irreversible process that alters the tooth structure, meaning once the tooth is sectioned, it cannot be restored to its original form. [9]

2. Loss of Surface Features

The grinding process may lead to the loss of fine surface characteristics like enamel texture, which are important for studies involving wear or dental caries.[20]

3. Complexity in Interpretation

Ground sections provide a two-dimensional view of the tooth's internal structures, making it difficult to interpret the 3D anatomy accurately. This can complicate studies focused on the entire structure or spatial relationships within the tooth.[21]

4. Artifacts from Cutting and Grinding

The cutting and grinding process can introduce artifacts or distortions, potentially leading to misinterpretations in the analysis of tooth structure.[22]

5. Time-Consuming and Labor-Intensive

Preparing ground sections is labour-intensive and requires specialized equipment and skill, making it more time-consuming compared to other non-destructive techniques like micro-CT imaging.[7]

6. Inability to Examine Soft Tissues

Ground sectioning is limited to hard tissues such as enamel and dentin, making it unsuitable for studying soft tissues like the pulp, periodontal ligament, and other surrounding structures.[12]

7. Limited Quantitative Analysis

The method may not be as effective for detailed quantitative analysis (e.g., measuring microstructural features or mineral density) compared to modern non-destructive imaging techniques like X-ray microtomography.[23]

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