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Histological Comparison of Ground Section, Decalcified Section and Resin Embedded Section of Bone- A Review.

Godlin Jenita
Undergraduate student
Department of Prosthodontics
Saveetha Dental college,Saveetha Institute of Medical and Technical Sciences
Chennai

Gheena S
Reader
Department of Conservative and Endodontics
Saveetha Dental college,Saveetha Institute of Medical and Technical Sciences
Chennai

DhanrajGanapathy
Professor and Head
Department of Prosthodontics
Saveetha Dental college,Saveetha Institute of Medical and Technical Sciences
Chennai

CORRESPONDING AUTHOR:

DhanrajGanapathy
Professor and Head
Department of Prosthodontics
Saveetha Dental college,Saveetha Institute of Medical and Technical Sciences
Chennai
Email:dhanrajmganapathy@yahoo.co.in

Abstract
In Ground section there is no need to remove the mineral, so that the bone in the section contains both the mineral and the collagen that form most of the bone structure. Resin embedding can make immunohistochemical antigen detection or DNA isolation for clonal gene rearrangement assays difficult. It gives better nuclear staining and reduces hydrolysis but may damage the tissue. In decalcified section inorganic substance is dissolved and the organic substance remains while in ground section organic substance is burnt and inorganic substance remains. This review is done to compare the ground section, decalcified section and resin embedded section of the bone to determine the better processing for microscopical study of the bone.

Keywords:bone,groundsection,histochemistry

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Introduction:

Histological examination of hard tissues using light microscopy techniques requires a standardized thin section procedure[1]. Histochemistry examination of living tissue cannot be conducted because it is difficult to maintain integrity of the tissues (hard tissue and soft tissue) in such condition. Therefore fixing the tissue is important first step for any histological processing and sectioning of tissues [2]. reservation of hard tissues is essential for understanding of cellular and sub-cellular structures and functions. Bone and bone-containing specimens are inherently difficult tissues to work with histologically[3]. Problems arise in cutting sections of bone marrow biopsy specimens, bone tumour samples, or biopsies of metastases to the bone because of the intimate mixture of hard tissue (bone) and soft tissue (marrow, fat, or neoplastic tissue). To cut adequate intact sections, one can either make the tissue uniformly hard by freezing of fresh material or by embedding in plastic, or one can make the tissue uniformly soft by decalcification followed by paraffin embedding[4]. The histological examination is based on the organic and inorganic content present in the sample after processing and sectioning of the hard tissue[5]. Hard tissue involves both teeth and bone of the body.

In this review, articles based on histological preparation of Ground section, decalcified section and resin embedded section of human bones were compared to come to a conclusion of which method is more acceptable and easier to study histology of bones.

Fixation

Fresh tissue at normal room temperature becomes liquified with a foul odour . This is because of changes occurring in the tissues due to putrefaction and autolysis. The aim of the fixation is to preserve the tissue components to do this in such a way as to allow for the preparation of thin, stained sections [2]. It preserves the structure of the tissue by providing mechanical strength and stability to the treated materials. Fixatives alter the tissues to increase the integrity of the cell.

Fixation is the first and foremost step in a multistep process to prepare a sample of biological material for microscopic examination. Types of fixation are heat fixation, immersion and perfusion. Chemical fixatives are used in immersion and perfusion. Neutral buffered formalin (NBF) is the normal chemical fixative used for paraffin embedded sectioning [6]. This is equivalent to 4% paraformaldehyde in a buffered solution plus a preservative (methanol) which prevents the conversion of formaldehyde to formic acid. Formalin is the common fixative used for any method of sectioning and preservation.

Methodology for Ground section:

Decalcification of bone and teeth leads to obscuration of some structures. The equipment used for ground section of hard tissue includes Saw, Forceps laboratory lathe, a fine and coarse abrasive lathe wheel, water directed onto rotating wheel, wooden block, adhesive tape, brush, ether, mounting medium, microscopic slides and cover glass, Scalpel, Reagents including 50% alcohol, xylene and DPX [7,8]. The cut surface of the hard tissue (teeth) is grounded using coarse abrasive lathe wheel followed by fine abrasive lathe wheel. The tissue was ground down to a thickness of about 0.5 mm and the surface is made smooth using fine abrasive lathe wheel. The finished ground section was soaked in ether for few minutes and

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dried for few minutes. The ground section of the tissue should not be let to dry for more time because it may lead to cracking [7]. A constant spray of water as well Paris powder is required to be sprayed on grinding surface while grinding. Paris powder prevents irregular grinding of tooth and water help to cool as tooth get heated up due to friction of grinding[9]. The transverse bone section was placed on the flint paper, which was kept on a stable flat surface. After the section becomes transparent which is of 25 – 30 micron , grinding was stopped and then the section was washed under running tap water. The section was viewed in the microscope and the clear part was trimmed using scalpel and forceps. The tissue was then mounted with thick DPX and cover slip avoiding air bubbles [8]. Hard tissue (teeth) was embedded in the plaster slab and trimmed using modern trimmer with water for cooling. The tooth was ground down to approximately 4- 5 mm thickness and then transferred to bench and grounded for 2-3 mm. Then it is coarse abrasive is replaced by fine abrasive and trimmed to 1mm For polishing and removal of irregularities Slurry of pumice and water was then rubbed with the section on Arkansas stone to make it smooth and paper- thin [10]. And then it is mounted on the slide using DPX solution and cover slip. The femur of the human bone was cut by a handsaw in a diaphyses section. Four different method was used. The transversal sections were ground by wet SiC13 – Method 1, by carborundum - method 2, by carborundum after submergence of the cross sections into methyl methacrylate solution for 24 hours - method 3, and by carborundum again after application of a few drops of cyanoacrylate glue onto periosteal surface of bone and on the surface made by the first cut - method 4. After grinding, the sections were glued onto standard glass microscopic slides and covered with cover glass. After covering with glass, the sections were slowly warmed up (carbonization). After grinding, sections were put into distilled water with a few drops of detergent, air–dried, sealed off into hot Canada balsam and covered with m–xylene dipped cover glasses. After grinding, the sections were washed, air- dried, placed into 95% ethanol, air-dried again and finally coated by methyl methacrylate solution [11].

Methodology for decalcified section

Decalcification i the technique of removing mineral from bone or other calcified tissue so that good-quality paraffin sections can be prepared that will preserve all the essential microscopic element. Decalcification is carried out after the specimen has been thoroughly fixed and prior to the processing to paraffin. There are three main types of decalcifying agents- strong mineral acids, weaker organic acids and cheating agents [12]. Electrical method is also nowadays used for decalcification of tissues [13].

After fixation of the specimen the decalcification is followed. The specimen should be placed in the test tube and is suspended in about 400 ml of 5% nitric acid. The acid should be changed daily for 8- 10 days and it is tested for complete decalcification. The end point of decalcification is difficult to assess but it is important. Then the decalcified specimen must be washed for 24 hours to remove the acid. After washing, the specimen should be dehydrated with increasing percentage of alcohol for 24 to 48 hours. Then the specimen should be infiltrated with parlodion. Parlodion is purified nitrocellulose dissolved in ether alcohol. The specimenis transferred to 2% parlodion and then to increasing percentage of parlodion. The time taken for infiltration depends on size of the material and amount of bone and tooth material present. After infiltration with parlodion, more parlodion is added so the thickness is

about 13 mm of parlodion above the specimen. The specimen is then oriented in proper plane for cutting. The disk was then covered with a lid to permit slow evaporation of ether alcohol. As it is evaporated, the parlodion solidifies. Hardening takes for at least 2 – 3 weeks. After this, the block was sunk in chloroform and then changed to 70% of alcohol for removal of chloroform. And the specimen is sectioned using sliding microtome and then stained. A modification of this embedding technique is using acid celloidin instead of parlodion. This preserves organic matrix of tooth enamel during process of decalcification [7].
One group of bone was exposed to 50 ml of hydrochloride acid of different concentrations of 0.4, 0.2, 0.1, 0.05, 0.025 N. Other group, 1gm is exposed to tetrasodium salt of EDTA
(ethylenediaminetetraacetic acid). Then other 5gm of bone was exposed to disodium salt buffered with phosphate to 7.4 ph [14].

Methodology for resin embedded section

Hydrophilic resins minimise the denaturation of proteins.
For resin embedding, fixative is glutaraldehyde (4% in 0.1 M phosphate buffer). Fixation is followed by dehydration with alcohol and then alcohol is replaced by resin in infiltration. Excess resin is trimmed before sectioning. For sectioning, glass or diamond knives are preferred. Dry sectioning is preferred for epoxy resin whilst wet is better for methacrylate [15].
Composite resin can also be used which should be cured for 60s [1].
The samples were dehydrated in a graded ethanol series and then infiltrated three times for 40 min with withlowicryl and bioacryl at right temperature., As a catalyst, 1.3 g of benzoyl peroxide was added to the mixture, which was then carefully sealed and gently stirred until the catalyst had dissolved. Both Lowicryl- and BothLowicryl- and bioacryl-infiltrated samples were polymerised in Ep- pendorf tubes by UV light for 72 hr at 4’C. The embedded specimen were sectioned using ultra microtome and then subjected to various histological stainings [16].
JB-4 resin component A 9g, Methyl methacrylate monomer 1g and Dried benzoyl peroxide 45mg were used as an infiltrating solution . Infiltrating solution (pre-chilled) 10g and JB-4 resin component B 250µ were mixed and used as an embedding solution[17].

Histology of ground section, decalcified section and resin embedded section
Ground section of bone (ulna) under low power magnification shows osteons containing concentric lamellae, A Haversian canal in the centre of each osteon, Lacunae in between the concentric lamellae containing osteocytes, Volkmann’s canals connecting haversian canals in a ladder rung-like fashion, Interstitial lamellae in the intervals between haversian system [8]. Enamel, dentin and cementum can be examined using the thin ground section of tooth [10].
Ground section made by bycarborundum again after application of a few drops of cyanoacrylate glue onto periosteal surface of bone and on the surface made by the first cut, shows beautiful sections microscopically on magnification x 200. Differentiated secondary osteons and some of them connected with Voklmannscannals are seen. lot of interstitial lamellae in between the particular osteons were also seen in the sectional microscopic view. But the outer circumferential lamellae was not identified [11].

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. Organic substance are removed from the ground section hence the inorganic structures can be seen clearly in ground section while the organic structures can be seen in decalcified section.

Resin embedded section shows better morphological details with little shrinkage of bone marrow cells. Osteoclast can be demonstrated well o all periosteal and endosteal bone. Osteoblasts and leukocytes were demonstrated[18].

Comparison:

Resin embedded technique allows preservation of the fine structure and have been widely used in routine ultra structural studies because of their shrinkage and low stability in the electron beam [16]. Acrylics have provided a suitable alternative to epoxides to provide immunological information because there are so many ways of combining such monomers, an almost endless number of embedding media can be prepared [16]. Hydrophilic resin also reduces denaturation of proteins [19]. Ground section of bone is comparatively hard to prepare than other two methods but it is the most suitable for research of historical human bone tissue microstructure [11]. If the bone section becomes too thin there will be nothing left to look at; if it is too thick, the structures near the surface will be confounded by features that are buried inside [21]. Foot filer for coarse grinding of bone section can be used but flint paper produces more smooth sections [8]. The main advantage of ground section is that it is simple, cost effective and time saving. It equivalently has disadvantage which is Cracks or distortion of the sections may occur during grinding and all the soft tissues are removed during this procedure [10]. Bone decalcification is a time-consuming process. It takes weeks and preservation of the tissue structure depends on the quality. Shrinkage of pulp away from dentinal wall in teeth may be affected by fixation and processing techniques as well as by decalcifying agents[3]. But in contrast EDTA does not alter the integrity or molecular structure of the bone [14]. The decalcification of condyles in nitric acid, formic acid and EDTA took 22, 33 and 57 days, respectively for complete decalcification. It is a time consuming procedure compared to other two method.

Conclusion

All the methods are comparatively useful because of their own advantages. Recently resin embedded technique is been used widely. It gives immunological information in addition to pure morphology.

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