Essentials Before Sending Biopsy Specimens: A Surgeon's Prespective and Pathologists Concern

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Abstract Pathology is a science, which deals with the scientific study of structure and function of the body in disease. It involves both macroscopic and microscopic study of the tissue correlating it with the clinical and radiographic history, thus helping to arrive at an accurate diagnosis. Proper history and clinical description of the excised specimen has to be conveyed to the pathologist. More than proper surgical technique is required to facilitate the proper diagnosis of an oral biopsy specimen. The proper preparation of the tissue for microscopic analysis depends on steps taken by the surgeon, assistant, and histotechnician to reduce the inclusion of artifacts. This article particularly deals with various requirements for proper handling and transportation of specimens, important things which are to be communicated to pathologists, and various methods used to preserve the tissue for use in latest techniques.

Keywords Histo-pathology · Tissue fixation · Oral biopsy

Introduction

As the dental profession moves toward additional emphasis in detection of disease and sophistication in diagnosis, biopsy is being used with increasing frequency as a diagnostic tool [1]. Biopsy makes it possible to confirm or deny a diagnosis, as well as to determine the nature and characteristics of the lesion and to establish a final diagnosis.

Many cases, an inadequate gross dissection and sampling or iatrogenically induced artifacts will invalidate the microscopic interpretation. The dissection, gross description and selection of sections for microscopic study is a crucial part of the pathologic examination, and one that often cannot be remedied if omitted or done poorly at the time of the initial work up.

More than proper surgical technique is required to facilitate the proper diagnosis of an oral biopsy specimen. The proper preparation of the tissue for microscopic analysis depends on steps taken by the surgeon, assistant, and histotechnician to reduce the inclusion of artifacts. Biopsy specimens removed from the oral cavity are often small, and there is high potential for causing artifacts at several stages: during removal, fixation, embedding, or staining [2, 3].

So to make the best possible outcome from the sent tissue for histopathological examinations and to minimize the artifacts, here are some of the tips, which may greatly yield in surgical practice. Such familiarization in turn will contribute to knowledge of the material and instruments required for correct biopsy performance in dentistry, as well as of the material required for correct sample storage and transport.

This article is divided into three sections for better understanding of the topic. These are divided as follows.

1. Things to be taken care for during and after the biopsy surgery
2. During fixation and transport and
3. Important information to be communicated to pathologists

Things to be Taken Care for During and After the Biopsy Surgery

First of all, most importantly, surgeons should take adequate care to avoid contamination of the tissue with

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extraneous material, particularly tissue from another patient. This may happen in the operating room or clinic or during any of the steps of the pathology procedure at the time the tissue is examined.

Specimens removed from the oral cavity are often small, and the possibility of producing artifact is thus enhanced. These artifacts are based on the procedure during which these occur as, Injection Artifact, Forceps Artifacts, Fulgeration Artifacts, Laser Artifacts, and Suction Artifacts [2, 4].

One of the most common, injection artifact is caused by injection of excessive amounts of anesthetic solution at too rapid rate resulting in separation of connective tissue bundles. To avoid this, infiltration of anesthetic agents directly into the lesion should be avoided. This is particularly more important in case of immune mediated mucocutaneous disorders [2].

Forceps artifacts results when the teeth of the instrument penetrate the specimen, resulting in voids or tears and compression of the surrounding tissue. The surface epithelium may be forced through the connective tissue producing small “pseudocysts”. Using small atraumatic or Adson forceps will produce little mechanical distortion of the tissue or a suture placed to the edge of the specimen may be used as a substitute.

The use of cautery for biopsies has led to the emergence of artifacts. Microscopically such tissue shows a coagulated and torn appearance that makes histological evaluation impossible in the cauterized areas. To avoid this only the cutting and not the coagulation electrode should be used while performing biopsy [4].

Another type of artifacts may be induced by the vacuum effect of the surgical suction tips most notably in the connective tissue around odontogenic cysts and dental follicles. These manifest by the formation of large often pleomorphic connective tissue vacuoles resembling traumatized adipose tissue [2, 4].

Regarding lymph nodes dissection, the first step consists of dissecting the node containing fat from the organ in fresh state, using forceps and sharp scissors. One should be gentle with the nodes at the time of the dissection as it is too easy to crush them especially if dissected before fixation. The lymph nodes should be separated and labeled in groups according to the type of the specimen and sent in separate containers. Also, when suspecting lymphoma in a patient, it is advisable to take an imprint of the dissected fresh lymph node on a clear glass slide, which can be used for imprint cytological techniques along with microbiological assessments [1, 3, 5].

Regarding margins one of the most important components of gross examination and sampling is the evaluation of surgical margins; under the assumption that a positive margin will likely lead to local recurrence if uncorrected. This is usually carried out by painting those margins with

India ink or a similar pigment before sectioning; this can be done on either the fresh specimen or after fixation by gently wiping the margins with gauze and carefully covering the entire surgical surface with India ink using a cotton swab stick [5].

During Fixation and Transport

As Culling has stated “As much damage may be caused to a specimen by leaving it dry as by immersing it in an inappropriate fixative”. Process of autolysis and bacterial attack start as soon as tissue is removed from the body. So the first aim of fixation is to arrest these changes [6].

Fixation differs for different groups of chemical substances found in tissues. More importantly, it should be done as soon as possible after removal of tissue with an effective and appropriate fixative. The amount of fixative should be 15–20 times the bulk of tissue to be fixed and must surround the specimen on all sides. Tissues can also be left in fixatives for overnight for primary fixation to take place. Large specimens that float on the fixative should be covered by a thick layer of gauge and in cases they rest on the bottom of the container the gauge should be placed between the container bottom and specimen [6, 7].

Although fixation is used to prevent the occurrence of various artifacts, it may itself be the cause of various artifacts. The most common being volume changes, artifactual pigments, and diffusion artifacts. It has been found that, 33% shrinkage takes place in formalin fixed paraffin embedded specimen. In addition prolonged fixation in formalin has been found to give rise to secondary shrinkage. It is worthwhile to mention that normal saline solution gives almost no fixation [6–8].

Delay in fixation and inadequate fixation alters the staining quality of the cells. Cells appear shrunken and show clumping of cytoplasm. The nuclear chromatin becomes indistinct and nucleoli are sometimes not seen. The implications of this finding are in handling of oral biopsies especially of dysplasia and carcinomas [4].

For routine histopathology, fixation should be carried out at room temperature. For rapid fixation of urgent biopsy specimens, formalin heated to 60°C has been used. Formaldehyde at 100°C can be used to fix tissue infected with TB [6].

Hollow specimens like cystic cavities are either opened fresh or else fixed simultaneously from the outside and inside. Hollow specimen cavity is filled with formalin by syringe or catheter or packed with gauge or cotton impregnated with formalin. Cystic lesions are injected with formalin after the original fluid has been removed. Multilocular cysts require individual injection of larger cavities [5].

Apart from routine histo-pathology, various other techniques like Immuno-histochemistry, Enzyme-histochemistry,

Fluorescent microscopy, Electron Microscopy, exfoliative cytology etc. are finding their way in routine diagnosis; it becomes a need to exactly know what kind of different fixative needs to be used for these techniques [9, 10].

Special Circumstances

Electron Microscopy

To preserve ultra-structure of cell, fix tissue as soon as possible. If delay in fixation is unavoidable, specimen should be chilled, but not frozen, preferably in normal saline. Routine fixation is done in two steps, by primary fixation with an aldehyde (usually glutaraldehyde) precooled to 4°C to stabilize proteins, followed by secondary fixation in osmium tetra-oxide (OsO₄) to retain lipids [6].

Immuno-Fluorescence, Immuno-Peroxidase and Histochemical Techniques

Routinely tissues are processed on cryostat machines, so the tissue may be used fresh (after air drying) or fixed in cold 95% alcohol, absolute methanol or acetone. Fixation must be limited for few seconds [10].

Exfoliative Cytology Smears (Brush Biopsy)

After obtaining the cells on glass slide, either spray the surface of slide with commercially available alcohol fixative or else dip in fixative solution like 95% ethyl alcohol, which is universally used. The cells will degenerate if they are allowed to air dry [6].

It is worthwhile to mention that if, tissues have already been fixed in routine manner with formalin, then also, there are special technique to use such tissue for all the above mentioned techniques by processing them in a special manner [6, 7].

Regarding transport of specimen, it should be done in appropriate sealed containers and should be as soon as possible. If delay is unavoidable, it is better to communicate to pathologist and to ascertain with him the type of tissue and proper fixative required. The container should have an opening large enough so that the tissue can be removed easily after it has been hardened by the fixation.

Important Informations to be Communicated to Pathologists

Always tissue has to be sent with a proper requisition form, given an identification number and stating all relevant clinical details like site, size, shape, dimensions etc. Dimensions as seen clinically and after tissue has been

excised has to be mentioned clearly as tissue may swell up or shrink in fixative. Also some specimens like cysts may loose its contents while in transport [1, 3, 6].

It is also essential to communicate the urgency for report as histopathology reports can be sent in as small time as like ten minutes (by use of cryostat) to one day or may also take sometimes few weeks periods, if there is requirement for special tests or if specimen contains any bony tissue. Like frozen section technique is usually employed for ensuring margins free of malignancy in case of head and neck dissections [3, 6].

A common mistake while taking gross photographs is to take a photograph of the external surface of intact tumor but omitting photograph of cut surface which is more informative. For color photographs a gray toned neutral intensity color is preferable (light-blue). Use of drapes, sponges and gauzes is to be discouraged. Whenever possible normal structures should be included in the photograph to serve as a frame of reference for the lesion.

With historical background, physical findings and precise orientation of anatomic relations, the pathologist can block the tissue in the plane that will give meaningful sections. If microscopic description is inadequate, the slide can be reviewed and the problem may be corrected.

Any previous reports of same patients if performed somewhere else may also be added upon. In many surgical excisions the surgeon already knows the microscopic diagnosis of the lesion and he is now interested in informations such as extent of lesion, invasion of neighboring structures, presence of tumour at surgical margins, vascular invasion and lymph node metastasis.

Conclusion

So from above discussion it is very clear that, if we take some extra little care for tissue, as we do for the patients, by following some of the tips as mentioned above, it can definitely be a great help for the patient, the pathologist and the surgeon in rendering a good diagnosis and a good treatment.

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