Clinicians’ role in the occurrence of oral biopsy artifacts as a potential diagnostic dilemma

Rola lekarzy klinicystów w powstawaniu artefaktów w badaniu histopatologicznym jamy ustnej jako możliwy dylemat diagnostyczny

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Abstract

Lack of knowledge about biopsy techniques and management of biopsy specimens can cause artifacts. Artifacts are false structures that change the normal morphological and cytological features of tissues. This review aims to familiarize the clinicians/dentists/surgeons with the factors causing artifacts and with the efficient strategies to prevent or minimize their occurrence. Non-adherence to several rules can result in the formation of artifacts. The clinician’s performance during and after the surgical procedure (until the sample is received by the laboratory) may damage the biopsy specimen or make it susceptible to damage. Artifacts may not be clinically considered noteworthy. However, by modifying the histopathological features of the specimen, they can lead to serious errors in the interpretation of lesions. Knowledge on the part of clinicians/dentists/surgeons regarding the factors and potential conditions that can lead to artifacts can decrease risk of their occurrence and considerably help the pathologists and patients by paving the path to make a correct diagnosis and consequently suggest an appropriate treatment plan.

Key words: biopsy, dentist, artifact, oral lesion

Słowa kluczowe: biopsja, dentysta, artefakt, zmiana patologiczna w jamie ustnej
**Introduction**

Detection of oral lesions by dentists is highly important,\(^1\) because in many diseases or conditions, oral lesions manifest sooner than cutaneous lesions.\(^2\) This can help in early diagnosis and subsequent management of the disease in its initial stages.\(^3\) Clinical diagnosis of lesions is confirmed with different techniques.\(^1\) The gold standard of diagnostic procedures is biopsy.\(^4\) Biopsy is defined as taking a sample of a living organism,\(^4,5\) which can include the entire lesion or a part of it.\(^5,8\) Biopsy is performed to allow a histopathological analysis of the specimen under a microscope to make a definitive diagnosis.\(^9,10\) This assessment is important for diagnosis of lesion and also to determine the presence/absence of evidence of malignancy. Biopsy provides information about the clinical course of lesion and may even provide prognostic data. All these can directly affect patient management.\(^11,12\)

Nonetheless, in some cases, dentists fail to send tissue specimens to histopathological laboratories and explain that they are concerned about the risk of misdiagnosing the lesion.\(^12\)

One challenge in the correct interpretation of histopathological sections is the presence of defects not related to the existing disease.\(^13,14\) In microscopic analysis, such defects can create manifestations that are not necessarily related to the actual histological/histopathological features of the specimen.\(^13\) These defects are known as artifacts.\(^13\) The term ‘artifact’ is derived from 2 Latin terms: ‘ars’ and ‘factum’ and is defined as a false or altered tissue structure in a microscopic feature as a result of some external factors.\(^5\) Artifacts are illusive structures, although they can be misinterpreted as real ones.\(^13\) Artifacts can interfere with the histopathological assessment of the specimen\(^6\) and affect the pathologists’ ability to correctly diagnose lesions, especially in small samples.\(^12\) They also compromise the correct histopathological interpretation of specimens, especially at the margins.\(^15\)

Biopsy is a simple, minor surgical procedure.\(^16,17\) However, achieving an accurate and correct diagnosis requires more than just correct surgical technique.\(^18,19\) Suitable tissue preparation for microscopic analysis depends on the correct performance of the dentist/surgeon, assistant and lab staff to minimize the risk of artifacts occurring.\(^19\)

Biopsy (taking a tissue sample) has 6 steps: selection of biopsy site, preparation of surgical field, anesthetic injection, incision, handling of biopsy sample, and suturing of the surgical wound.\(^20\) Processing of tissue sample is a long process, from surgical removal to staining and mounting of tissue sections on the slides. Artifacts may occur at any step, from the time of taking biopsy to the final step of mounting.\(^19\) It is imperative for clinicians/dentists/surgeons to have adequate knowledge about the artifacts\(^5\) and this study is aimed to familiarize them with tissue artifacts. This review article discusses the causes of artifacts during surgery, fixation and transfer of samples to the lab in order to find strategies to minimize their occurrence.

**Preoperative surgical artifacts**

Artifacts can change normal morphological and cytological appearance of the biopsied samples.\(^5,21\) They may be confined to only a small portion of the sample. In these cases, they can be easily detected by an experienced pathologist and no longer interfere with an accurate pathology report and a correct diagnosis.\(^5\) However, in some cases, the artifactual damage may be extensive or involve the entire sample. In such cases, artifacts result in suboptimal quality or quantity of the sample for diagnosis or may even render the tissue useless.\(^21\)

**Artifacts caused by the surface preparation of the surgical field prior to biopsy**

After the biopsy site has been precisely selected,\(^1\) preparation of the site with iodine tincture or other colored solutions should be avoided.\(^5,22\) Colored antiseptics or similar agents are not recommended for disinfecting the surface or the external margins of the incision site\(^6\), since they can interfere with tissue processing and staining procedures. If they were nevertheless used, dentists must inform the lab about it.\(^21\) It should be noted that toluidine blue, used to determine the most representative part of premalignant and malignant oral lesions,\(^3,23\) does not interfere with the aforementioned processes.\(^6\)

**Artifacts caused by injection**

Injecting the anesthetic solution into the biopsy site can damage the tissue sample\(^6,8\) and cause 2 major tissue changes\(^24\):

- bleeding and extravasation of red blood cells due to needle insertion into the tissue and subsequent possible masking of tissue structures\(^7,24,25\);
- splitting of connective tissue bands associated with vacuolization.\(^20,24\)

In microscopic feature, area in which anesthetic agent has been directly injected shows artifactual tissue edema or distortion, which can lead to misdiagnosis. For instance, formation of a bulla or artifactual edema in the gingival tissue may mistakenly lead to the diagnosis of Crohn’s disease or orofacial granulomatosis.\(^26\)

Block injection is recommended to prevent artifacts. If not possible,\(^7\) the site of infiltration anesthesia injection should be adequately far from the lesion.\(^25\) Injection should be administered with at least 3–4 mm distance from the lesion and at 4 points around it (superior, inferior, right, and left).\(^7\)

Excessive pressure should be avoided\(^7\) and the anesthetic solution should be injected slowly.\(^19\) If achieving hemostasis is among the objectives of the injection, it should be administered deep into the lesion or postponed to immediately after the biopsy.\(^25\)
Artifacts during surgery

Artifacts due to the use of biopsy instruments

Some cutting instruments such as a scalpel, punch, forceps, electrode blades/electroscalps, and laser blades are used to take biopsy samples.\(^7,^{15,27}\)

Scalpel

Biopsy samples are conventionally obtained using surgical scalpels.\(^3,^{11}\) This is considered a conventional method of biopsy.\(^3,^{11,28}\) It seems that an incision with a sharp scalpel causes less tissue damage.\(^3,^{14}\) Sharp instruments are required for a biopsy.\(^3\) The sample should be taken with 1 sharp incision.\(^3\) Multiple incisions with a blunt scalpel\(^3,^{15,22,28}\) can cause a number of artifacts in the sample.\(^28\) A blunt scalpel can cause squeeze artifacts,\(^22\) which are a form of tissue distortion that can be caused even by the slightest compression of tissue.\(^3,^{20}\) These artifacts include crush, hemorrhage, split, fragmentation,\(^5,^{20}\) or occurrence of pseudocysts in the tissue (Fig. 1A–E).\(^5,^{20}\)

Split artifact can occur at the surface or margins of samples taken with scalpel (especially a blunt scalpel).\(^3,^{28}\) They are caused by several incisions made with the scalpel, resulting in a split between the epithelium and the connective tissue (Fig. 1F).\(^5,^{28}\) The split artifact creates a false impression of vesiculo-bullous lesions.\(^5,^{28}\)

Crush artifact is destructive and dangerous,\(^5,^{24}\) and can occur even with the slightest compression of tissue.\(^5\) It changes the tissue morphology and squeezes the chromatin out of the nucleus.\(^24\) Microscopically, crushed cells appear as black chromatin strands, which may be mistaken for dysplastic lesion.\(^5\) Inflammatory and tumor cells are most susceptible to crush artifact (Fig. 2A–C).\(^24\)

Forceps

They can also be used to perform a biopsy.\(^7,^{27}\) They facilitate the biopsy of oral soft tissue and small salivary glands. Forceps have 2 cusps and a window that allows compaction of target tissue between them.\(^7\) The use of dentate forceps is an old technique. They firmly hold the tissue and acceptably protect it during removal.\(^26\) If used inappropriately, forceps can also cause squeeze artifacts just like blunt scalpels.\(^5,^{22}\) Forceps should never grasp the lesion\(^25\) because they can cause crush artifact due to tissue grasping.\(^3,^{8}\) Dentate forceps can create holes in the tissue when used carelessly with high force.\(^3,^{16}\) These holes can histologically mimic mucosal pits or an epidermoid cyst.\(^16\) Inappropriate use of forceps can also result in the formation of pseudocysts in the tissue.\(^3,^{16,24,25}\) The teeth of the forceps can push superficial epithelium into underlying connective tissue. This, along with the compression of connective tissue, can create pseudocysts.\(^3,^{24,25}\)

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Fig. 1. A. Epithelial crush artifact. B. Connective tissue crush artifact. C. Fragmentation of tissue sample. D. Hemorrhage artifact. E. Split artifact. F. Split between epithelial and connective tissue, false impression of a vesiculo-bullous lesion. H&E staining; original magnification x100
These pseudocysts, which apparently line with the surface epithelium, can make the exact assessment of the sample difficult.5,24,25 When the teeth of forceps perforate the tissue, they not only create voids or tears, but also compress the surrounding tissue.19,25 Microscopically, the tissue appears distorted with scalloped serrations (caused by the forceps tip) and crushed cells (Fig. 3A–C).5,25 Compression of tissue eliminates its cytological details. It also changes the dimensions of the nucleus and particularly its ratio to the cytoplasm.24,25 These changes may be sufficient to complicate the diagnosis16 or lead to a misdiagnosis.25 Forceps artifacts can be overcome by correct handling and careful use of this instrument.5

The lesion should not be directly grasped with the forceps;25 the grasped tissue should be acceptably far from the biopsy site.26,29 Blunt forceps should be used instead of toothed forceps.13,24 Excessive force at the time of grasping should be avoided in order to avoid significant changes in the epithelium and underlying connective tissue.25 Suturing should be performed at the sample border and used instead of forceps for tissue immobilization.24

**Punch**

The use of punch is an affordable, fast, simple, and safe technique for biopsy.7 Evidence shows that it creates less artifacts in the tissue compared to using a scalpel.13,15 In punch biopsy, artifacts are in the form of tissue fragmentation, probably due to the use of scissors for separating the sample from the underlying tissue base.13 It cannot be used for deep lesions and punch biopsy is limited to epithelial or superficial mesenchymal tissues.7 The use of punch in some areas such as soft palate, maxillary tuberosity or floor of the mouth is difficult due to the mobility of site and absence of a hard, fixed tissue.3,7 Punch biopsy of freely mobile tissues3 can damage the tissue and cause artifacts.3,7
Electrical cautery instruments and laser

Surgical electrical cautery instruments and laser have advantage of causing hemostasis during surgery. On the other hand, overheating generated by these devices can cause changes in the epithelial and connective tissues (fulguration artifact). In tissue sections with fulguration artifact, epithelial cells appear detached. The nuclei of cells have a spindled, palisading shape and are hyperchromatic. These changes can mimic the appearance of epithelial dysplasia and lead to an incorrect histopathology report of the lesion, especially at the margins, which are extremely important for the clinician in terms of presence/absence of dysplasia and invasion. In fulguration artifact, the separation of the epithelium from the basement membrane can be seen, which may be associated with epithelial loss. The underlying tissues such as fibrous connective tissue, fat and muscle have an opaque amorphous appearance. Protein is deposited when the generated heat results in the tissue fluids boiling. Microscopically, this tissue has a coagulated and torn appearance. In the lesion(s), particularly along the surgical margin(s), tissue protein coagulation in the form of a wide, extensive basophilic coagulum band is seen, giving an amorphous appearance to the epithelium and connective tissue (Fig. 4A–C).

To prevent this, it has been recommended that these instruments should be used according to the dysplastic potential of lesions. For lesions suspected of dysplasia or neoplasia, their use should be avoided or the incision margin should be sufficiently far from the interface of lesion and normal tissue. The use of a laser should be limited to excisional biopsies and relatively large samples where adequate margin is available.

Low power settings of the laser should be used to effectively decrease the risk of separating the epithelium from the basement membrane and of epithelial loss during tissue processing.

A combination of electrosurgery and scalpel should be used instead of electrosurgery alone, because the former would yield more favorable results. In this technique, the scalpel is used for the primary incision around or within the lesion and then the lesion is completely excised using electrosurgery. This method results in optimal hemostasis and minimizes heat exposure of sample.

Cutting electrodes should be used for tissue sampling rather than coagulation electrodes. This is conventionally done by using low, milliampere current.

Intentional or incidental contact of the tip of cutting electrode with metal instruments used for holding the sample should be prevented, because it can cause tissue changes.

Artifacts due to the improper handling of tissue specimens

Care must be taken when handling mucosal biopsy samples since the tissue specimen can be easily damaged. The presence of artifacts in histopathological sections of damaged tissue can decrease their diagnostic accuracy or render them useless. Traumatic injuries and rough handling can cause artifacts in the specimen. Suturing is one common method to decrease them. It enables traction and elevation of tissue during biopsy. Moreover, it prevents unwanted movement of tissue (especially mobile structures such as...
the tongue) during the procedure.\textsuperscript{26,29} They orientate at least one side of the lesion, facilitate the surgical procedure and prevent compression and destruction of tissue specimen.\textsuperscript{7} However, care must be taken since excessive traction of tissue can cause laceration or crushing of the specimen and significantly damage the epithelium or connective tissue.\textsuperscript{7} It can also split the epithelium from the connective tissue and mimic the appearance of vesiculo-bullous lesions.\textsuperscript{5} Forced traction of the surface epithelium against underlying connective tissue can cause pseudocysts and result in the loss of cytoplasmic and nuclear features.\textsuperscript{21} Excessive traction can cause tissue fragmentation\textsuperscript{28} and subsequent bleeding can be interpreted as a pathologic change by an inexperienced pathologist.\textsuperscript{13}

Thus, it is important:

- to handle the tissue specimen with care\textsuperscript{21,29};
- not to apply too much pressure during tissue traction with sutures\textsuperscript{5};
- to ensure that the sutures have not been placed within the designated area for biopsy\textsuperscript{5};
- to ensure that the suture knots are loose, since firm knots can cause tissue crush\textsuperscript{26} (inflammatory cells\textsuperscript{28} and tumor cells are susceptible to crush artifacts, which can make the specimen uninterpretable).\textsuperscript{24}

**Vacuum artifact**

This type of artifact is caused by vacuuming the tissues,\textsuperscript{9} especially the connective tissues around odontogenic cysts and dental follicles, with surgical suction tips. These artifacts are seen as large and mostly pleomorphic vacuoles in the connective tissue, similar to traumatized fat tissue.\textsuperscript{19}

**Artifacts due to the contamination of specimens**

Some artifacts are due to the contamination of the specimen with foreign bodies, which may occur during surgery.\textsuperscript{21,30} Cotton and starch are among the most common contaminants.\textsuperscript{20} Starch artifacts may occur due to contamination of specimen with starch powder, which is used as a lubricant in surgical gloves.\textsuperscript{5,20,21} Starch granules may be superficially similar to atypical epithelial cells\textsuperscript{5,20} and mimic the appearance of salivary gland diseases, autoimmune disorders, granulomatous lesions, or benign epithelial lesions.\textsuperscript{5} These spore-like structures with dark central area could be mistaken as pyknotic nuclei or mitotic nuclei.\textsuperscript{20,21} Starch granules appear light blue on hematoxylin and eosin (H&E) staining, blue-black with Lugol’s solution and deep lilac-red on PAS staining.\textsuperscript{5,20} Starch artifact can be prevented by using latex gloves.\textsuperscript{7} The presence of cotton in histopathological sections – cotton contamination – may resemble eosinophilic, amyloid-like or black substances.\textsuperscript{5,20} Similarity of cotton to amyloid-like material, which is among the characteristics of odontogenic tumors, may result in a misdiagnosis.\textsuperscript{5}

To control bleeding during surgery, gel foam or surgical sponges are used.\textsuperscript{13} In histopathological sections contaminated with a gel foam, the characteristic appearance of distorted superficial spaces is seen. These spaces may be filled with blood and surrounded by slightly basophilic gelatin walls.\textsuperscript{13} Dentists/surgeons should be careful to prevent foreign body contamination of specimens.\textsuperscript{19} On the other hand, knowledge about these materials and their correct identification in sections can help in establishing an accurate pathologic interpretation.\textsuperscript{5}

**Post-surgical artifacts (occurring after the specimen removal until a transfer to the lab)**

**Artifacts due to a delay in fixation**

After tissue removal, biopsy specimen should be fixed.\textsuperscript{5,7,17} Autolysis and bacterial attack initiate immediately after tissue removal.\textsuperscript{19,32} Fixation stops these changes.\textsuperscript{19,32,33} It maintains the integrity of the cells and their chemical components\textsuperscript{34} to preserve the tissue in conditions like when alive.\textsuperscript{13,34} The time interval between surgical removal of the sample and its immersion in the proper fixative is referred to as the ischemia or hypoxia time,\textsuperscript{34,35} which is associated with activation of tissue enzymes, autolysis and degradation of proteins, DNA and RNA.\textsuperscript{34} Delay in fixation yields a poor histopathologic feature.\textsuperscript{5} It also changes the quality of staining of cells\textsuperscript{24,25} and mitosis would be hard to detect.\textsuperscript{5}

Cells shrink and cytoplasmic clumping is seen.\textsuperscript{7,25} Chromatin of the nuclei cannot be detected and nucleoli are not visible in some cases.\textsuperscript{5,7} Some changes occur in tissue structures as well.\textsuperscript{5,7,25} Vascular, nervous and glands structures lose their details\textsuperscript{5,7,25} simulating scar tissue formation\textsuperscript{25} or loss of cellularity.\textsuperscript{5,7,25} To prevent the occurrence of these changes, biopsy samples should be placed in a suitable fixative immediately after removal.\textsuperscript{19,36}

**Artifacts due to the type of fixative used**

The 10% neutral buffered formalin is an alternative fixative to optimize the fixation of a biopsy specimen to routine histopathological assessment.\textsuperscript{12,24,36} Despite attempts to find a replacement for it,\textsuperscript{35,37} formalin remains the most popular, reliable and affordable fixation solution.\textsuperscript{34}

Tap water,\textsuperscript{3} distilled water or saline have been occasionally used for tissue fixation.\textsuperscript{18,20,33} Tissues fixed with these solutions often show abnormal cellular or structural changes\textsuperscript{33} that can pose a challenge for correct diagnosis of lesions.\textsuperscript{33} Saline does not cause any fixation at all\textsuperscript{19} and should not be used even for a short time.\textsuperscript{33} Immersion of specimens in saline causes bizarre appearance of epithelial cells, which can suggest malignancy.\textsuperscript{5} Antiseptics,
mouthwashes or local anesthetic solutions are also inappropriate for tissue fixation. The specimens cannot be properly fixed with these solutions and thus, tissue autolysis continues. Fixatives that are protein precipitants such as ethanol and methanol (irrespective of their osmotic pressure) cause tissue shrinkage. Thus, tissues that are attached to each other when alive may be pulled apart in these fixatives and leave empty spaces. Alcohol makes the tissue fragile and creates artifacts following microtome sectioning (chattering and a Venetian blind artifact). The use of ethanol as a fixator can cause crush artifact, which is due to protein coagulation seen as intense eosinophilic staining at the centers of tissue specimen in H&E staining. Ethanol does not completely fix proteins. The use of alcohol as a fixative results in weak staining of the epithelium and inadequate fixation of connective tissue. In these conditions, collagen bundles have an amorphous feature, which is not due to scar tissue formation; it is an artifact. When encountering a tissue specimen in an unsuitable fixative, it should be immediately replaced with 10% neutral buffered formalin to prevent further morphological changes in the tissue. Freezing the specimen before or instead of fixation is not suitable. It can cause dehydration of cells and subsequent condensation of cytoplasm. Formation of ice crystals following freezing of tissue specimen results in the formation of interstitial and intracytoplasmic vacuoles. In histopathologic sections of frozen tissues, gaps in the tissue cause the appearance of Swiss cheese holes in the epithelium, indicative of areas where ice crystals have perforated the tissue.

**Artifacts caused by formalin**

The 10% neutral buffered formalin is the best tissue fixative introduced so far. Fixation with formalin prevents autolysis and occurrence of some artifacts. However, some considerations should be taken into account when using it. Formalin itself can cause tissue artifacts. The concentration of formalin should be suitable to ensure adequate fixation. Formalin is a diluted solution of buffered formaldehyde. However, sometimes the ancillary staff may over-dilute it. This can result in weak fixation of tissue specimen. Artifactual changes occur in the form of acantholysis of epithelial cells while the basal cell layer remain attached to the underlying connective tissue. This acantholytic artifact can mimic pemphigus, as well as Hailey–Hailey or Darier’s disease.

To ideally preserve the morphological details of the tissue, fixation should be done at room temperature. Although the rate of fixation, overheating of formalin can cause vacuolization, over-staining of cytoplasm and appearance of pyknotic nuclei. The tissue specimen fixed in 10% neutral buffered formalin should be as soon as possible sent to a lab. Formalin fixation is often associated with color change, volume change (33% shrinkage), and hardening of tissue. Moreover, secondary shrinkage may occur in the specimen following long-term immersion in formalin. Similar to delayed or inadequate fixation, long-term fixation also causes some changes in the tissues. Long-term formalin fixation can cause secondary shrinkage and hardening. This can result in tissue separation microscopically mimicking empty spaces.

Formalin fixation can cause pigmentation artifact. The bonding of formalin with heme of red blood cells forms formalin–heme complex, which is seen as black precipitates in the tissue specimen. Fast transfer of formalin fixed specimen to the pathology lab enables the lab staff to start tissue processing within the optimal fixation conditions. Preservation of specimens depends on both the pathologist and dentist. Unfortunately, it seems dentists believe that the lab would start processing on their specimen immediately after receipt; however, that is not really the case.

**Artifacts due to the size of a specimen**

The ability of oral pathologists to correctly interpret a biopsy sample depends not only on the quality but also on the quantity of the sample. In biopsies, and more commonly incisional biopsies, if the specimen is thin, especially if it is in the form of a thin, delicate oral mucosa ribbon, its shrinkage in formalin causes curling and bending of tissue. This change, known as the curling artifact, complicates the orientation of specimen. Unsuitable orientation can lead to tangential incisions of the specimen or sectioning of only the epithelium or connective tissue and not both.

Moreover, curling of the specimen onto itself during fixation often results in the loss of junction between the epithelium and connective tissue, especially if the specimen does not have the submucosal layer or the underlying muscle.

This artifact can be prevented by ensuring adequate depth of specimen. If the biopsy sample is small, thin and narrow, the specimen should be immediately placed on a piece of stiff sterile/clean paper for a short time (i.e., 1 min) before its immersion in fixative such that its surface faces upward and its connective tissue side faces downward. By doing so, the epithelium is preserved in its correct position and we can ensure that the tissue specimen is positioned straight during fixation and is well-oriented for histopathological assessment.

Surgeons can use sutures to help in the correct orientation of the specimen and send it along a note regarding the relationship of suture(s) with the specimen to the lab.
Conclusions

Knowledge on the part of clinicians/dentists/surgeons regarding the factors and potential conditions that can lead to artifacts can decrease risk of their occurrence and considerably help the pathologists and patients by paving the path to make a correct diagnosis and, consequently, suggest an appropriate treatment plan.

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