Type of article : Review

Artifacts in Histopathology – A Review

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Abstract

Histopathological examination is considered to be the gold standard technique for making a definitive diagnosis of various human body lesions. The handling of tissue specimens consists of extensive procedures from the stage of surgical removal to the microscopic parts stained and placed. Owing to defective procedures, defects are normal in the tissue parts. These defects are known as artefacts. In histopathology artefacts may cause serious errors and misdiagnosis. The precision of the histopathological diagnosis is based on removing or reducing the artifacts in histopathological section.This article discusses the common artefacts found during slide analysis alongside the remedial steps that can be performed to distinguish between an artifacts and the constituent tissue.

Keywords: *Artifacts, Errors, Histopathology,Diagnosis*

Introduction

Histopathology is a science purely focused on microscopic examination and interpretation[1].Sometimes the existence or modification of a foreign material in tissue details may cause confusion and lead to incorrect or inconclusive interpretations. Such entities or modifications are commonly called "artefacts"[1]. They occur in tissue sections prior to fixation, during fastening, specimen grossing, tissue processing, sectioning, staining and tissue preservation[2]. Some artefacts can be easily differentiated from normal or diseased components of tissue, while others are difficult to differentiate from certain entities[2].They are the primary source of diagnostic problem[3]. This article is therefore intended to encourage understanding of the various common artefacts that can be found in histopathology, to provide a guide for their identification, to clarify their causes and, where possible, to suggest the means by which their occurrence may be avoided[2].

Depending on the stage at which artefacts are formed, they can be categorised as artefacts produced during[3,4]:

- Artefacts during oral biopsy procedure
- Prefixation artifacts
- Fixation artifacts
- Tissue processing
- Embedding
- Microtomy
- Mounting
- Staining
- Cover-slipping[4].

ARTIFACTS DURING ORAL BIOPSY PROCEDURE:

Artifact	Causes:	Remedies:
INJECTION	Intralesional injection of anesthetic solution3.	a) Use block technique instead of infiltration2. b) Avoid injecting larger amount of anesthetics4. c) Avoid injecting anesthetic adjacent to the biopsy site4.
FORCEPS AND CRUSH/ SQUEEZE:	a) Instrument penetrates the instrument resulting in voids and tear5. b) Marks on specimen created by forceps pressure4.	a) Use small forceps or adson's forceps without teeth1. b) Use B forceps for to take oral biopsy5. c) Handle the specimen gently and avoid compression or retraction of tissues2.
FULGERATION:	Heat generated while doing laser or electrosurgical procedures2.	Use knives, use low milliamperage current, use of combination knife and electrical points2.
CURLING:	a) Occurs in incisional biopsies5. b) Occurs due to shrinking process of oral mucosa after introduced in formalin5.	a) After the biopsy, the specimen is placed with its mucosal surface up on a piece of the sterile paper, allow the specimen to unfixed for sometime5. b) For thin specimen – take adequate depth of specimen to prevent this artifact5.
SPLIT:	Penetration of forceps into the tissue5.	Use blunt forceps instead of sharp or toothed forceps4.
SUTURAL :	Presence of suture material in histopathologic tissue2,7.	Visible sutures are removed wherever possible2,7.
STARCH:	Due to contamination of starch powder in tissue3.	a) Avoid touching of tissues by using gloved hands2,3. b) Use correct tissue forceps to prevent this artifact2,3.

FIXATION ARTIFACT:Fixation is a mechanism that attempts to maintain the tissues by prevention of autolysis and putrefaction in a life like environment[4]. The fixative volume should be 20 timesthat of the specimen not exceeding 6 mmthick[4]. 10 per cent of formalin is commonly used as a fixative[6]. Fixation objects emerge from formalin, mercury chloride, and picric acid used in various fixative agents that cause widely dispersed brown black granular and yellow stains in the tissues[2].

ARTIFACT:	CAUSES:	REMEDIES:
FORMALIN PIGMENTS:	Formaldehyde has a tendency to be oxidized, producing formic acid. Heme from RBCs and formalin bind each other to form formalin–heme complex that appears as brown-black amorphous to microcrystalline granules in tissue sections[3].	Removed from tissue section by immersing it in saturated alcoholic picric acid[3]. This can be prevented by using buffered neutral formalin[3].
MERCURIC PIGMENTS:	Produces dark brown granular deposits[3].	Removed by immersing it in alcoholic iodine[3].
ICE-CRYSTAL ARTIFACT:	Slow freezing of tissue due to selection of large sample to permit rapid freezing[3].	It can be prevented by using carbon-di-oxide expansion freezers[3].
FREEZING ARTIFACT:	Characterized by formation of interstitial and intracytoplasmic vacuoles resulting from ice-crystal formation taking place as 10% formalin freezes at -11°C[2].	Prevented by avoiding before fixation[5].
STREAMING ARTIFACT:	diffusion of unfixed material to give false localization by coming to rest in same place other than its original location3.	It can be prevented by using glycogen fixatives or by freeze drying[2,3].
SHRINKAGE ARTIFACT:	During fixation, tissues change its original size[2]. This occurs due to inhibition of cellular respiration and changes in membrane permeability[2].	It can be avoided by using compound fixatives[2].
DELAYED FIXATION:	Results in cell shrinkage and cytoplasmic clustering[5].	Fixing the specimen in 10% formalin immediately after tissue removal[5].
DIFFUSION ARTIFACT:	Due to materials that diffuse out of tissue[5].	Proper fixation for exact localization and also preventing leaching of ions from the tissue[5].

PROLONGED FIXATION: Prolonged fixation in formalin may cause secondary shrinkage and hardening and it may result in separation of tissue, giving an appearance of empty spaces[2,5,7].

POST FIXATION: Tissue that are fixed in chrome if not washed for 24 hours in running tap water could produce chromeoxide pigment[2,7].

MICROWAVE FIXATION: Optimum temperature for microwave fixation is 45-55 oC. Low heating results in poor section quality whereas overheating above 65oC results in vacuolation, overstained cytoplasm and pyknotic nuclei2.

TISSUE PROCESSING ARTIFACTS:

Artifacts during dehydration:

Artifacts can be encountered during dehydration because of

· Improper dehydration gradient, if the concentration gradient between the fluid within and outside the tissue is high, the current of diffusion crosses the cell membranes during the exchange of fluids, thereby increasing the probability of cell distortion[2,7].

· Over dehydration renders the tissue hard, fragile and shrunken, which causes difficulty when cutting and also interferes with sectional staining properties[2,7].

· Low and incomplete dehydration results in inadequate penetration of the rendered paraffin and block is difficult to section so that distorted sections of broken tissue can lead to artifactural changes2,7

Artifacts during clearing :

· Due to over-hardening of tissue specimens, objects can occur and obstruct paraffin to properly impregnate in paraffin wax, making it difficult to cut during sectioning[2,7].

· Artifacts can result from under and over clearing of tissue specimens and blocking the paraffin to properly impregnate in paraffin wax, making it hard to cut during sectioning2,7.

REMEDY: This can be avoided by taking good care to use the correct amount of clearing agent and there should be no clearing agent left behind to contaminate the wax[5].

Artifacts during impregnation:

· The purpose of wax impregnation is to extract the clearing agent (wax solvent) from the tissue and to allow them to be fully impregnated by the paraffin wax which is subsequently allowed to harden to create a block from which parts can be cut[2,7].

· The artefact produced during this procedure is crystallisation: the thicker the tissue the more clearing agent it holds, and thus more wax change is needed to remove it[2,7].

· Even if a small quantity of clearing agents contaminates the wax it will cause tissue to crumble and crystallise during cutting[2,7].

EMBEDDING:

ARTIFACT:	CAUSES:	REMEDIES:
INCORRECT ORIENTATION:	Exposing the specimen too long during embedding which results in hardening, so that it becomes friable and crack easily3.	Correct orientation of tissue5. Expose the specimen to correct amount of medium5.
AIR ENTRAPMENT:	Air gets entrapped while embedding which results in venetian blind effect4.	Care must be taken while orienting in the medium

ARTIFACTS RELATED TO MICROTOMY: (causes and remedies)

1. Alternative thick and thin section[1]:

CAUSES:	REMEDIES:
Wax too soft for tissue or conditions	Make it harden by using ice pack or re-embed with wax or highest melting point
Block or loosen knife	Tighten the knife
Insufficient clearance angle	Increase the clearance angle slightly
Mechanism of microtome faulty	See for any obvious faults

2. Thick and thin zones parallel to knife[1]:

CAUSES:	REMEDIES:
Knife or block in the holder is unfit or loose	Tighten
Steep knife angle (excessively)	Decrease the clearance angle to minimum
Tissue or wax is too hard for sectioning	Use sharp knife and microtome, decrease slant angle of knife, use softening or loosening fluid on tissue
Calcified areas in tissue	Rehydrate and decalcify

3. Curved ribbons and consecutive section[1]:

CAUSES:	REMEDIES:
Unparalleled leading and trailing edges	Sharp the scapel until parallel
One area of knife is blunt	Use other part of knife or sharpen it
Surplus area of wax at one side	Remove away the excess wax
Tissue varying in consistency	Orient the block again by turning through 90 degree, cool the block with ice.

4. Splitting of sections at right angles to knife edge[1]:

CAUSES:	REMEDIES:
Nick in edge of the knife	Use other part of knife or re-sharpen it
Hard particles in tissue	If calcium deposits are there, do decalcification, if mineral or other particles are present remove the particle by using scapel
Hard particles in wax	Re-embed the tissue in fresh and filtered wax

5. Section will not join to form a ribbon[1]:

CAUSES:	REMEDIES:
Wax- too hard for sectioning	Dip the block to warm or re-embed in wax at lower melting point
Debris on edge of the knife	Clean with xylene – dipped cloth
Knife angle (too shallow or too steep)	Change the optimal angle

6. Section become attached to block on return stroke[1]:

CAUSES:	REMEDIES:
Clearance angle is insufficient	Increase clearance angle
Knife edge containing wax debris	Cleaned by using xylene dipped cloth
Debris on edge of the block	Cut or trim the edge with scapel

7. Areas of tissue in block not present in section[1]:

CAUSES:	REMEDIES:
Impregnation of tissue incompletely	Tissue returned back to vaccum impregnating bath for few hours
Wax block detached from wood	By using hot spatula re-attach the wax block

8. Excessive compression of section[1]:

CAUSES:	REMEDIES:
Blunt knife	Sharpen the knife
Too wide bevelled knife	Re-sharpen to create secondary narrow bends

9. Section expand and disintegrate on water[1]:

CAUSES:	REMEDIES:
Poor tissue impregnation	Tissue returned back to vaccum and impregnating bath for few hours
Too high water temperature	Cool

10. Section rolled up into a tight coil instead of remaining flat on knife[1]:

CAUSES:	REMEDIES:
Blunt knife	Sharpen
Too small rake angle	Resharpen to create cutting angle or decrease knife tilt if clearance angle is excessive
Thick section	Decrease the tissue section

ARTIFACTS OF STAINING AND MOUNTING:

A) Stain Particles: Absorption of stain particles occurs when the staining chemicals are not sufficiently dissolved or the stain is not fresh, resulting in precipitation[1].

REMEDY: Remove or filter the stain

B) Air Bubbles: These are trapped over the stained portion when the cover slip is pulled on. These appear as minute spherical features[1].

REMEDY:

· Using sufficient quantities of mounting media[1].

· If more viscosity has been obtained with the mounting paper, xylene can be used as a thinning agent[1].

MISCELLANEOUS:

· Folds in sections: Folds on the wax pieces may often not be straightened resulting in folds or pleats. This is a typical artefact in hard tissue component tissues and is difficult to prevent even with the utmost care[1].

· Sectional cloudy appearance: Defective dehydration technique results in sectional cloudiness[1].

Conclusion

Tissue artifacts can be introduced into tissue specimen during any one of the many steps through which a specimen is carried before its microscope features are examined by the pathologist. Most of these artifacts may not be intentional and might go unnoticed causing pitfalls in diagnosis.Hence, proper handling of tissue along with prompt fixation and careful tissue processing will minimize the artifacts.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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