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Comparative Evaluation of Tissue Samples When Stored in Different Feasible Storage Media

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ABSTRACT:
INTRODUCTION: We all know that 10% formalin is the gold standard fixative for tissue storage during histopathological examinations, which helps preserve cells in a more lifelike state and preserves the integrity between the cells and the extracellular substances in the tissue sample. So, it is mandatory to store tissues in formalin for a proper examination and diagnosis. Often, dentists do not have formalin readily available in their dental clinics during biopsy procedures. In such instances, some commonly available storage media can be used as a transitional storage medium, and the tissue samples should be transferred to formalin as quickly as possible to avoid artifacts. The nature of the different feasible storage media and the storage time for tissue samples in each medium are examined and discussed in the following study.
AIM: To histopathologically evaluate the tissue samples when stored in different feasible storage media periodically.
OBJECTIVE:To evaluate Cellular Integrity,Basal cell layer,Cellular Architecture, Collagen fibres,Nucleusin the epithelium of tissue samples when stored in different storage media in a periodic interval.
METHODS:Tissue specimens are collected. The baseline PH of the storage mediums are evaluated (preferably maintained at Neutral PH). The collected tissue specimens are stored in three Eppendorf tubes containing Normal Saline, Saturated Saline and HBSS Solutions and 10% Formalin kept in a tube as a positive control solution. The tissue samples are evaluated for any artefacts at different periodic intervals.
EXPECTED OUTCOME: HBSS and Saturated Saline can be used as a ready-to-use tissue preservative for maintaining cells in a stable state for a limited period of time and should be transferred to formalin as soon as possible**.**

I. INTRODUCTION:

During Biopsy procedures, tissue is removed from the body to detect any pathological condition. To have a successful pathologic evaluation, a high-quality sample must be submitted. In enzyme-rich tissues, autolysis is a major issue, since autolyzed tissues are not stained properly for proper histopathological examination. We all know that 10% formalin is the gold standard fixative for tissue storage during histopathological examinations, which helps preserve cells in a more lifelike state and preserves the integrity between the cells and the extracellular substances in the tissue sample. So, it is mandatory to store tissues in formalin for a proper examination and diagnosis. Often, dentists do not have formalin readily available in their dental clinics during biopsy procedures. In such instances, some commonly available storage media can be used as a transitional storage medium, and the tissue samples should be transferred to formalin as quickly as possible to avoid artifacts. In this study, HBSS, Saturated Saline, and Normal Saline are used as intermediate media to store tissue samples. The nature of the different feasible storage media and the storage time for tissue samples in each medium are examined and discussed in the following study.

II. MATERIALS AND METHODS

Materials planned to be used for the study:
Tissue samples, Conical and Eppendorf tubes, Formalin, HBSS, Saturated Saline, Normal Saline, Gauze Packs, Light Microscope, BP blades, Eosin and hematoxylinstains,etc

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Methodology:

Tissue specimens are collected.

The storage media's baseline pH is assessed and, ideally, kept at neutral pH.

The collected tissue specimens are stored in three Eppendorf tubes containing Normal Saline, Saturated Saline and HBSS Solutions.

An extra sample of tissue preserved in a tube containing a 10% formalin solution as a positive control.

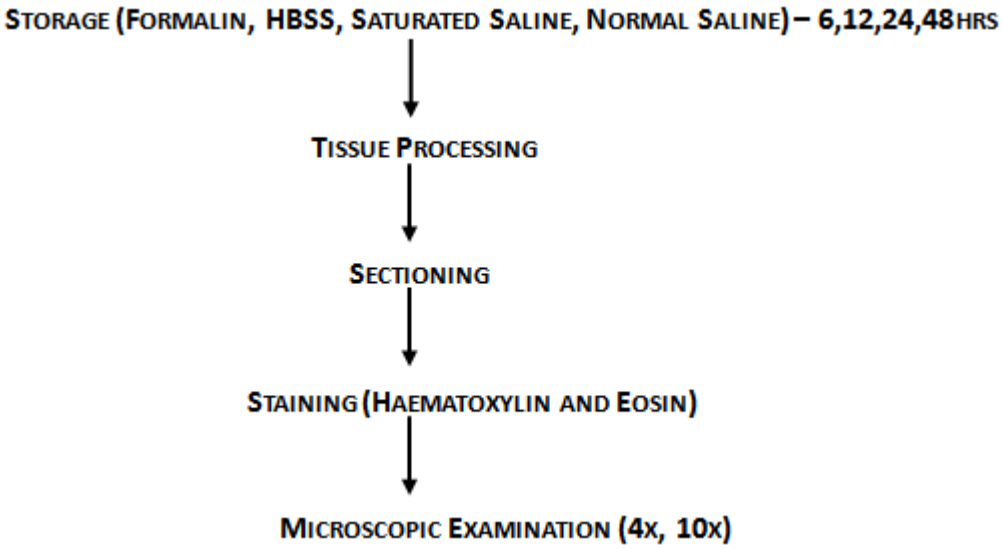
Periodically, the tissue samples are inspected for artefacts.

Each specimen underwent standard histopathological processing, and thin slices were obtained of thickness.

Harris' Haematoxylin and Eosin was used for the staining process in the histopathological examination.

The tissue samples are evaluated forCellular Integrity,Basal cell layer,Cellular Architecture, Collagen fibres andNucleus.

Photographic comparison of these tissues with their typically fixed control was done.



III. RESULTS :

NORMAL SALINE:

After one hour in normal saline, a tissue sample revealed cell vacuolization in the basal cell layer.Cellular integrity was lost at 12-hour intervals.Tissue left in regular saline for 24 hours showed non-vital, anucleated cells. Total cellular architectural lysis occurred after 48 hours in the standard saline solution, making the tissue non-diagnostic. [1]

SATURATED SALINE:

After 24 hrs the Saturated sodium chloride solution preserved the tissue and cells without causing them to grow or shrink, nor did it change or dissolve the components of the cells.There were no morphological (swelling or shrinking) abnormalities and the intact normal histological section architecture was almost identical to that of the Positive control group(formalin).

HANKS BALANCED SALT SOLUTION:

HBSS effectively preserves cellular activity and morphology for limited periods, typically a few hours to 24 hours. The solution's pH balance (around 7.0-7.4) is critical for preventing degradation in epithelial tissues and maintaining structural integrity. For tissues with epithelial layers, periodic immersion in HBSS can support cell preservation and delay apoptosis and necrosis compared to unbuffered saline solutions, which may lack pH regulation and essential ions.HBSS is generally not suitable for extended storage periods beyond 24 hours, as it lacks the necessary nutrients and proteins found in more complex culture media.[15]

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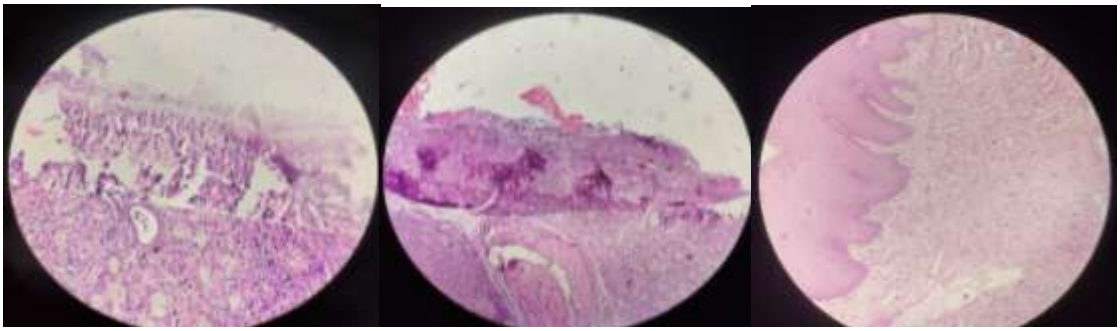
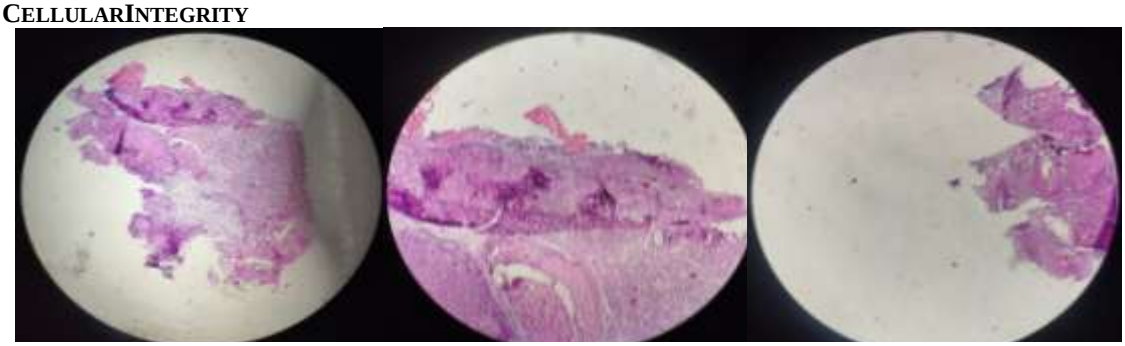
HISTOPATHOLOGICAL SLIDES:

1. **CELLULAR ARCHITECTURE**



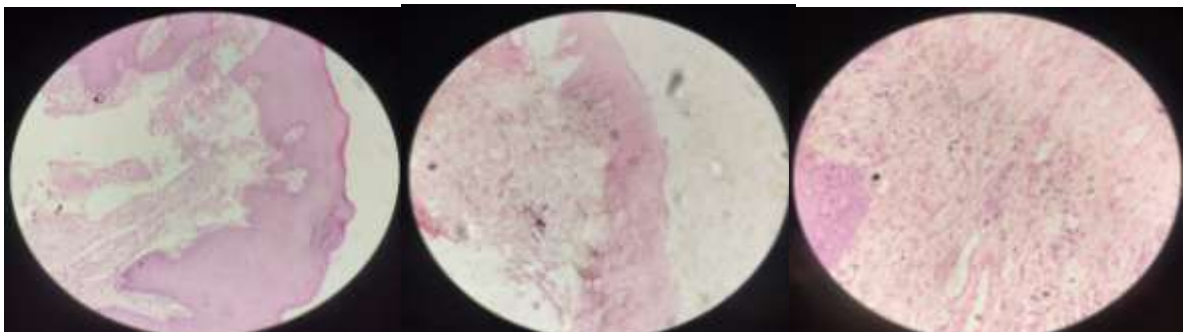
NORMAL SALINE **SATURATED SALINE** **HBSS**

2. **CELLULARINTEGRITY**



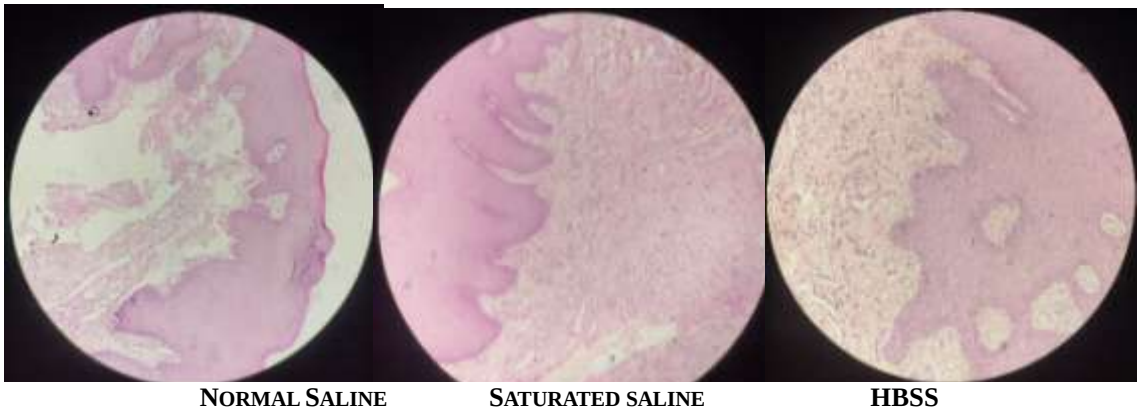
NORMAL SALINE **SATURATED SALINE** **HBSS**

3. **CONNECTIVE TISSUE FIBRES**



NORMAL SALINE **SATURATED SALINE** **HBSS**

4. **BASAL CELL LAYER**



NORMAL SALINE **SATURATED SALINE** **HBSS**

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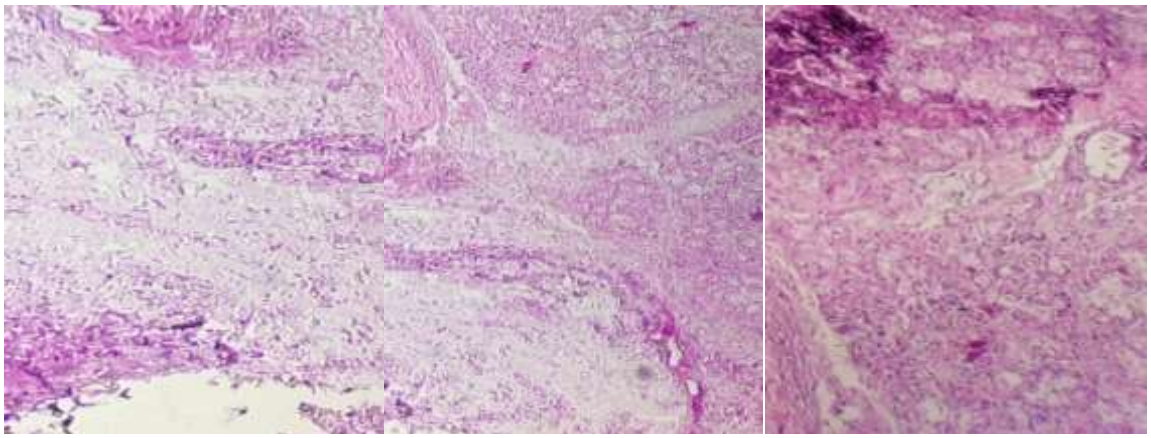
5. NUCLEUS



NORMAL SALINE **SATURATED SALINE** **HBSS**

IV. DISCUSSION

Preventing tissue necrolysis is necessary for a suitable histological evaluation. The enzymes secreted by the tissues themselves cause autolysis which leads to the destruction of labile substances in the sample. Hence the tissue samples are fixed in an appropriate fixative to prevent or arrest autolysis and maintain it in a lifelike state for a period of time. [1][2]

Fixatives are materials that try to keep tissues and cells in their natural life-like state and preserve components like enzymes, cellular architecture, nuclear morphology, and complex organization of the tissues. As a result, Fixation has been accepted as the primary tissue processing procedure.[1][3]

Fixatives function by many processes, which can be broadly classified as follows: Dehydrants, heat effects, cross-linkers, acid effects, and their combinations. Protein-combining agents are referred to as additives, and protein-precipitating agents are referred to as coagulants. It is now widely understood that no single fixative can achieve all of the objectives associated with the preservation of cells or tissues.[4][5]

Typically, 10% neutral buffered formalin is used to store tissue samples. The buffer's function is to stop the pH from falling too low, which could increase autolysis. [1][6] The pH of the fixative buffer significantly influences the degree of cross-linking. Hydrogen ions are released from the charged amino acids when neutral buffered formalin is used, as the pH is brought to neutrality. Further cross-links can be formed by the reaction of reactive hydrogen present in these uncharged groups with formalin. The application of 10% buffered formalin, therefore, tends to generate more cross-links than non-buffered formalin.[1][7] When producing (H and E) slides, formalin is utilized for all standard surgical pathology and autopsy tissues.[4]

Alternative forms of fixatives that are employed include coagulative fixatives, such as ethanol, which precipitate proteins by rupturing hydrogen bonds in the absence of protein cross-linking.The majority of bodily proteins have both hydrophilic and hydrophobic moieties. The removal of water with the usage of ethanol affects hydrogen bonding, which destabilizes the proteins. Thus, the precipitation of the protein's tertiary structure occurs.[7]

Fixation introduces a significant artefact on its own.The degree of distortion in the tissue caused by formaldehyde, specifically shrinking, is the primary concern.[8]

Previous research has shown that formaldehyde exposure can have major side effects and is strongly suspected of being carcinogenic to humans.When formaldehyde is first exposed, it might trigger an immunological reaction.Anyone exposed to an acute exposure may cough and wheeze in addition to experiencing extreme irritation of the eyes, nose, and throat. Subsequent exposure may induce severe allergic reactions of the skin, eyes and respiratory tract. Formaldehyde can be lethal if consumed, and prolonged exposure to low concentrations in the air or on the skin can result in respiratory conditions similar to asthma as well as skin irritation including dermatitis and itching.[9,10]

The introduction of an alternate fixative that is more appropriate for tissue preservation can help address formaldehyde's drawbacks.[4]According to a study, formaldehyde can be replaced with an inexpensive saturated table salt solution to preserve animal remains.[11]Morphological structures and immunoreactivity were found to be preserved when skin and lymph node fragments were fixed in

anhydric sodium chloride for weeks or months at room temperature.[12]Saturated sodium chloride solution can be employed as a fixative in histopathology procedures, according to Al-Saraj's research. The outcomes were identical to those achieved with traditional formaldehyde.[4]Saturated sodium chloride had the additional benefit of not distorting the cell. [13]The HBSS is a sterile, physiologically balanced isotonic standard salt solution, i.e., widely used in biomedical research to support the growth of many cell types. This solution is nontoxic; it is biocompatible.Hank's Balanced Salt Solution (HBSS) is commonly used as a storage medium for tissue samples in cell biology, histology, and other biological research applications.Due to its capacity to maintain PDL cell viability over an extended period of time, the American Association of Endodontists advises using HBSS as the preferred storage medium for the treatment of avulsed teeth.Even in cases where the extra-alveolar duration is lengthy (between 72 and 96 hours), it is a preferred storage medium for avulsed teeth.[14]

V. CONCLUSION:

Hence Hanks Balanced Salt Solution and Saturated Saline can be used as atemporary tissuestorage media for maintaining cells in a stable state for a limited period and should be transferred to formalin as soon as possible**.**

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