Wrinkle-free tissue sections
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Abstract
Aim: The aim was to compare the efficiency of routine method, isopropyl alcohol, and acetic acid solutions for removing wrinkles/folds from the tissue sections obtained.

Materials and Methods: Formalin-fixed paraffin-embedded tissue blocks of six oral tissues, two uterus (cervix) tissues, and two skin tissues were employed. Dilute isopropyl alcohol solution was prepared by mixing one volume of isopropyl alcohol with 15 volumes of distilled water, 30 mM and 40 mM of acetic acid were also used and were employed as flotation media.

Results: In our study, sections lifted from the water baths, after floatation onto all three different media, yielded largely similar results. In the case of oral tissues, alcohol seemed to give marginally better results.

Conclusion: Dilute alcohol and acetic acid are the alternate flotation media used in this study, but the control group gave comparatively better results.

Keywords
Acetic acid, isopropyl alcohol, tissue sections, wrinkles/folds

Introduction
Histotechnology is concerned with processing and preparing of the body tissues in such a manner so as to enable a satisfactory study of it. The presence of artefacts like fine wrinkles/folds is usually encountered in the routine method of preparing paraffin sections. Tissue sections without folds are essential for proper visualization and accurate diagnosis. Oral biopsies are usually small in size and contain a less keratinized surface making them more liable for folding artefact. The traditional methodology of floating sections in water also renders them more prone for folding due to the property of surface tension. Hence, in this study, dilute isopropyl alcohol and acetic acid were employed for tissue section floatation with the aim of assessing the ease of removal of tissue folds and wrinkles. The overall effect on tissue visualization was also evaluated.

Materials and Methods

Tissue sections used in the study
10 blocks of formalin-fixed paraffin-embedded tissue blocks were employed. The tissue blocks consisted of six oral tissues, two uterus (cervix) tissues, and two skin tissues.

Solutions used for analysis
Isopropyl alcohol: Preparation of dilute alcohol solution was by mixing one volume of isopropyl alcohol with 15 volumes of distilled water.\(^1\)

Acetic acid: Two concentrations 30 mM and 40 mM were taken.\(^2\)

Tissue preparation
Ribbons of 5 µ thick sections were obtained in a semiautomated soft tissue microtome (Leica). The ribbon was observed for any gross imperfections and in the absence of which, were divided into six parts. First part of the ribbon was stretched in the water and transferred to the water bath (tissue floatation bath) heated to 34-38°C, and the sections were taken onto an adhesive applied glass slide.

Second part of the ribbon was floated on the dilute alcohol solution for 3-5 min, and they were then transferred to the water bath and taken on to a slide.\(^1\)

Third and fourth part of the ribbon were floated on the acetic acid in two concentrations 30 mM and 40 mM, respectively, for 10 min after stretching in water for 1 min; then sections were taken on to an adhesive applied glass slide.\(^3\)

Fifth and sixth part of the ribbon were taken through the same concentrations of acetic acid but before lifting the tissue directly
from acetic acid, the sections were transferred to the water bath and then taken on an adhesive applied slide, (i.e.) 30 mM (W) and 40 mM (W).

Slides were deparaffinized and stained with routine H and E staining technique. The sections were observed under light microscope for the presence of folds or minor wrinkles. The slides were also analyzed for staining intensity and overall quality of sections.

Results

On the analysis, we found that among the oral biopsies 4 (40%) out of the six oral tissue sections floated on water had folds ranging from 1 to 10 in number [Figure 1]. The use of alcohol as a floatation agent gave us similar results with 5 (50%) tissue sections having <10-folds [Figure 2]. When the tissue sections were lifted directly from acetic acid media, the results obtained were less satisfactory than the tissue sections taken through water bath after acetic acid [Figures 3-6]. Surprisingly, the use of acetic acid resulted in a greater number of folds with around 50% of cases showing >20-folds [Table 1].

When the cervical biopsies were also taken into account and an overall comparison was done, the results obtained with the use of acetic acid 30 mM (W) and 40 mM (W) were comparable to that of the control and alcohol groups with 6 (60%) and 5 (50%) tissue sections, respectively, having folds between 1 and 10. Acetic acid 30 mM group had 4 (40%) sections with 11-20 folds, whereas acetic acid group at 40 mM showed the greatest number of folds ranging between 21 and 30 [Table 2]. The overall results obtained with the use of acetic acid as the floatation media were thus concluded as being unsatisfactory.

Discussion

Artefact refers to an artificial structure or tissue alteration on a prepared microscopic slide, which results due to an extraneous factor. Wrinkles and folding of tissue sections are caused due to very thin sections being unevenly stretched around other structures having different consistencies. If folds have occurred, they are usually removed by forceps or by transferring the sections to another water bath at high temperature as in routine method. Manually stretching the sections is technique

| Table 1: Frequency of number of folds seen in oral tissue sections |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Number of folds | Control (%) | Alcohol (%) | Acetic acid 30 mM (%) | Acetic acid 30 mM (W) (%) | Acetic acid 40 mM (%) | Acetic acid 40 mM (W) (%) |
| 1-10 folds | 4 (40) | 5 (50) | 1 (10) | 4 (40) | - | 3 (30) |
| 11-20 folds | 2 (20) | 1 (10) | 1 (10) | 1 (10) | 1 (10) | 3 (30) |
| 21-30 folds | - | - | 1 (10) | - | 3 (30) | - |
| >30 folds | - | - | 3 (30) | 1 (10) | 2 (20) | - |

| Table 2: Frequency of number of folds seen in overall tissue sections |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Number of folds | Control (%) | Alcohol (%) | Acetic acid 30 mM (%) | Acetic acid 30 mM (W) (%) | Acetic acid 40 mM (%) | Acetic acid 40 mM (W) (%) |
| 1-10 folds | 7 (70) | 7 (70) | 2 (20) | 6 (60) | - | 5 (50) |
| 11-20 folds | 3 (30) | 3 (30) | 4 (40) | 3 (30) | 2 (20) | 3 (30) |
| 21-30 folds | - | - | 1 (10) | - | 6 (60) | 2 (20) |
| >30 folds | - | - | 3 (30) | 1 (10) | 2 (20) | - |
sensitive and can result in a tear. Floatation in water can actually result in the causation of folds or minor wrinkles due to its high surface tension.\textsuperscript{[6]} Isopropyl alcohol and acetic acid, both readily available in any histopathology laboratory have a lesser surface tension than water. Hence, utilizing these fluids either as a replacement of water floatation or as an intermediary step was analyzed.

Alcohol sets up diffusion currents and also helps to reduce the surface tension in water that flattens the tissue sections.\textsuperscript{[5]} In a study done by Kumar \textit{et al.}, one volume of absolute ethyl alcohol (ethanol) was mixed with 15 volumes of water i.e. 1:15 dilution which was used as the floatation media for reduction of tissue folds and good results were obtained when compared with the normal routine method.\textsuperscript{[5]} Our study used a similar methodology using isopropyl alcohol as it is economical when compared to ethyl alcohol. With the use of alcohol as the floatation agent, 5 (50\%) out of six oral tissue sections had <10-folds thereby giving similar results [Figure 2, Table 1]. When overall tissue sections were considered, comparable results were obtained between control and alcohol groups. 7 (70\%) sections out of 10 tissue sections had wrinkles/folds <10 in number [Table 2].

Acetic acid also performs with the same principle of reduced surface tension and two dilution systems and two floatation methods were used. Study done by Ahsan \textit{et al.} used acetic acid of various concentrations and examined for the reduction in the tissue folds and immunohistochemistry was also done. They observed that with <40 mM concentration of acetic acid solution, wrinkles were seen in both epithelium and connective tissue zones and tissues treated with concentrations at 80 mM and higher showed cracking between the epithelial layer and lamina propria. Poor immunohistochemical staining was seen for cytokeratins 13 and 17, even though there were no wrinkles. The results concluded that 40 mM was the optimal concentration of acetic acid solution to prevent wrinkles and gave good quality immunohistochemical staining for oral tissue sections.\textsuperscript{[2]} In our study, only two concentrations were used i.e. 30 mM and 40 mM, and the result showed no reduction in the tissue folds with this floatation media. Using 30 mM concentration, only 1 (10\%) oral tissue showed folds <10 in number and 3 (30\%) oral tissues showed folds more than 30 in number. The 40 mM concentration showed that all oral tissues had folds more than 10 in number [Table 1, Figure 3 and 5]. Hence, we tried using water bath after lifting the tissues from acetic acid which gave better results i.e. 30 mM (W), 40 mM (W) concentrations showed 4 (40\%) and 3 (30\%) oral tissues respectively with folds <10 in number, as shown in Table 1 and Figures 4 and 6. When overall tissues were considered, similar results were obtained [Table 2].

Our study is the third such study done to use different floatation media to reduce the tissue folds obtained during routine method. In our study, sections lifted from the water baths, after floatation onto all three different media, yielded largely similar results. To obtain better results, the angulation of the slide to lift sections from the water bath should be standardized, and intense care should be taken for smaller oral tissues.

**Conclusion**

The proper handling of biopsy specimens is important and essential for histopathological diagnosis. Even with the recent advances in laboratory equipment, the need to recognize
sectioning artefacts and attempts to overcome them still remains a challenge. Thus, considering the caveat of a small sample size, we conclude that the use or additional floatation agents do not contribute to reduction in tissue folds as similar results were obtained with the use of floatation agents and routine techniques.

References
