PARAFFIN EMBEDDED TISSUE BLOCKS: BOON OR BURDEN

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Abstract

Background and Aim: This is an era of continuous research that warrants the instigation of new ideas and their effective implications. For decades, it is a common practice to preserve the paraffin-embedded tissue blocks after histopathological reporting. Somehow, very less evidence is available in the literature confirming the utility of the same. We hypothesize that sectioning of preserved blocks after a due period may show noticeable morphological changes. The present study was planned to compare subjective and morphometric changes in old and freshly prepared slides from the same paraffin-embedded tissue blocks.
Materials & Methods: 2 groups were formed, Group 1(old) and Group 2 (new). Group 1 consisted of old slides and Group 2 consisted of freshly prepared slides made from the same paraffin blocks. 100 archival Hematoxylin & Eosin-stained slides were randomly chosen from the year 2008 to 2011. 47 slides were selected and blocks of the same were identified and resectioned and fresh slides were prepared. These were subjected to standard subjective and objective analysis. Student paired "t" test was used.
Results: The observation and results of the subjective and morphometric (objective) analysis proved that there were no significant changes when the 2 groups were compared.
Conclusions: We concluded that there was hardly any change in the architecture of tissues of preserved blocks after 15 to 16 years.

Keywords: Histopathology; Morphometry; Paraffin

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1. Introduction

It is a well-known fact that Histopathology is the gold standard and conclusion for all diagnoses.[1] Histopathological diagnosis sometimes becomes very important in case of recurrences, referral (second opinion) and re-investigations.[2] For academic purposes, resectioning of preserved paraffin embedded blocks may be required for retrospective longitudinal research. It has been observed that dental and medical institutes across the world are taking pains to preserve formalin-fixed paraffin-embedded (FFPE) tissue blocks. This does not only make use of manpower but also occupies a lot of space in laboratories. This motivated us to find out whether stocking preserved tissue for a long period is worth the effort. Hence, we planned a study to compare archival and freshly prepared slides based on their staining quality, ability to diagnose, artifactual changes, and dimensional alterations. Archival FFPE blocks present an immediately accessible resource for carrying out various studies [3] including DNA extraction [4], RNA extraction [5,6], protein estimation [7], and immunohistochemistry.[9] To the best of our knowledge this is a pioneer study that assessed qualitative and quantitative analysis of archival and recently Hematoxylin & Eosin (H&E) stained slides obtained from the same paraffin blocks.

2. Materials & Methods

The present prospective study was carried out in the Department of Oral Pathology & Microbiology, People's Dental Academy, Bhopal, Madhya Pradesh. The institutional ethical clearance was obtained for the study. We randomly chose 100 archival H&E slides from the year 2008 to 2011. These slides were thoroughly viewed by 3 experienced Oral pathologists and the best slides with optimal epithelial thickness were selected. 47 slides were finally selected for the study. The corresponding blocks were found and were re-sectioned. This was followed by preparation of new slides and staining in the same manner. 2 Groups were made (Group 1-Old slides & Group 2-New slides). All the slides were assessed based on subjective and morphometric analysis. Subjective analysis- The parameter for subjective analysis was the staining intensity and artifactual changes. The categorization for staining intensity included very good, good, satisfactory and inadequate. The parameters observed were identification of nucleus and cytoplasm, cellular outline, uniformity in staining and intensity of colours for H&E. The artifactual changes included any significant shrinkage and were denoted as being present or absent. Morphometric analysis- This was done with the help of Magnified Image Projection System software attached to a trinocular research microscope (Olympus CX 41). The epithelial component of all 47 slides of Group 1 & Group 2 was evaluated and 3 areas were studied for difference in the thicknesses of the epithelium. The 3 regions studied were extreme left side of the slide, centre of the slide and extreme right side of the slide. They were measured and compared (Fig 1a and Fig 1b). The data collected was tabulated to obtain a mean value.

Statistical analysis

The data analysis was done using the statistical package of social sciences 25.0 software (SPSS Inc., Chicago, USA). The mean values and standard deviations were calculated. Results were statistically analyzed by using independent t- test. For all statistical purposes, a p-value of ≤ 0.05 was considered significant.

3. Results

In the present study, 47 old (Group 1) and new (Group 2) slides were evaluated subjectively and objectively. 53.19% of the freshly prepared slides (Group 2) showed better staining compared to 17.02% in Group 1 whereas only 10.63% in Group 2

showed inadequate staining when compared with 34.04% in Group 1 [Table1]. When artefactual changes were compared in 2 Groups, it was found that 14.89% in Group 2 showed changes as compared to 6.38 % in Group 1. This was followed by a morphometric assessment of epithelial thickness as shown in Fig 1a and Fig 1b. The mean value of each slide was tabulated. As a result, we found that the difference in the mean thickness of epithelium was not significant (p-value ≥ 0.05) [Table 3]. However, the diagnosis remained the same for all the tissues which were the main concern.

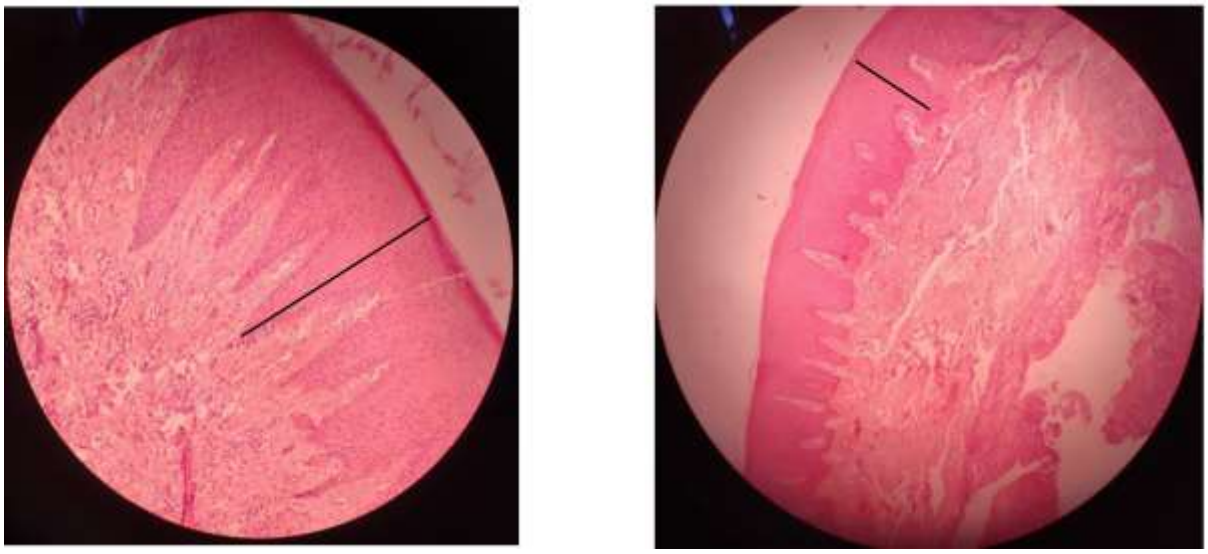

Fig 1a and Fig 1b- Representation of measurement of epithelial thickness in Group 1 and Group 2 slides

Groups	Very Good staining intensity (n %)	Good staining intensity (n %)	Satisfactory staining intensity (n %)	Inadequate staining intensity (n %)	Total (n=47)
Group 1	8(17.02%)	9(19.14%)	14(29.78%)	16(34.04%)	47(100%)
Group 2	25(53.19%)	10(21.27%)	7(14.89%)	5(10.63%)	47(100%)

Table 1- Comparison of staining intensity between Group 1 & Group 2

Groups	Present (n %)	Absent (n %)	Total (n=47)
Group 1	3(6.38%)	44(93.61%)	47(100%)
Group 2	7(14.89%)	40(85.10%)	47(100%)

Table 2- Comparison of presence or absence of artefacts between Group 1 & Group 2

Groups	Thickness of epithelium in extreme left side of the slide Mean± SD	Thickness of epithelium in the centre of the slide Mean± SD	Thickness of epithelium in extreme right side of the slide Mean± SD
Group 1	1705±550	1695±487	1725±548
Group 2	1710±567	1698±444	1730±598
t-value	0.032	0.006	0.021
p-value	0.974	0.995	0.983

Table 3- Comparison of morphometric analysis in the extreme left side, centre and right side of the slide.

4. Discussion

FFPE preserves the morphology and cellular details of tissue samples. This makes it a standard preservation procedure for diagnostic surgical pathology.[3] Histopathology is the master key to the diagnosis of dental and medical pathologies.[9] It has been observed that the pathologists take painstaking efforts to process and preserve formalin fixed paraffin embedded blocks. We were curious to know if it is worth taking the effort. Hence, we conducted a study wherein we compared old slides with newly made slides. On assessing the slides based on subjective criteria, we observed that the newly stained slides had better staining quality as compared to the old slides. This may be attributed to the fact that old slides tend to fade over some time.

Alteration in epithelial thickness may be due to the following reasons. Firstly, the three-dimensional structure of the tissue made it difficult to obtain similar histopathological sections. To overcome this limitation, we measured the epithelial thickness at 3 arbitrary levels (1^{st} at the extreme left side of the slide, 2^{nd} in the centre of the slide and 3^{rd} at the extreme right side of the slide), still, we found that there was an alteration in epithelial thickness. The results were not significant. Secondly, resectioning of archival tissue blocks led to the loss of initial ribbons which further induces changes. One of the drawbacks of the present study was that we have not included and measured connective tissue that has various elements such as blood vessels, collagen fibres, etc. So, it opens the window for future research.

5. Conclusion

Through our study, we concluded, that although there may be minimal non-cognizable dimensional changes, the overall picture and dimension remain the same, and hence blocks can be used in the future for many more years.

6. References

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