International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 26-28

Received: 14-11-2018
Accepted: 18-12-2018

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Scanning electron microscopy of paraffin embedded white pulp of spleen of goats

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Abstract
The paraffin embedded tissue sections and the cryofractured tissue obtained by conventional processing for electron microscopy are compared under scanning electron microscope. The routine processing of white pulp of the spleen procured from the slaughter of goats by paraffin embedding method revealed better results than conventional processing for electron microscopy. Epithelial reticular cells, T lymphocytes, B lymphocytes, plasma cells, dentritic cells and interdigitating cells were observed in both methods at scanning electron microscope. The clarity and contrast of depth of focus was better at magnification of 4000 in deparaffinized specimen. The paraffin processing method was simple and less expensive and combines the advantages of great depth of focus and high resolution of scanning electron microscope with the simple preparatory techniques employed for light microscopy than the conventional electron microscope processing techniques which is very expensive.

Keywords: white pulp, spleen, sem, paraffin, goat

Introduction
The conventional processing techniques for electron microscopy are too expensive and complicated. The routine processing of animal tissue by paraffin embedding method revealed better results than conventional processing for electron microscopy. In this study the tissue fixed with glutaraldehyde and processed using osmium tetroxide in phosphate buffer followed by treatment with uranyl acetate were compared under scanning electron microscope with that of formalin fixed and routinely processed tissue sections. Since scanning electron microscopy of tissue and cells are limited to the study of surface phenomena the paraffin embedded tissue sections after deparaffinizing proved to be better than special EM processing methods. White pulp of the spleen procured from the goats slaughtered for meat in Thanjavur were used as sample of study. Methods involving freeze fracture can be useful for exposing internal structures of tissues, but these techniques were often complex and insufficiently precise for observing and quantifying highly localized histological events [4]. Using SEM on section of paraffin embedded tissue combines the advantages of serial sectioning and differential staining for standard light microscopy with high magnification and increased depth of focus. Visualization of three dimentional orientations of both tissue and cells were made using less expensive paraffin processing.
This method allowed direct correlation of LM images with those obtained by SEM, thereby improved interpretation of scanning electron micrographs [1, 3]. The application of SEM to anatomical studies of paraffin embedded sections were demonstrated with human [2] and plant tissue [8] at a lower magnification of two thousand to two thousand five hundred, but present study demonstrated in animal tissue over four thousand times of magnification.

Materials and Methods
Small pieces of spleen tissue (half centimeter cube) were collected from the slaughtered goats in Thanjavur. Six samples were immediately fixed in 2% glutaraldehyde in phosphate buffer and were kept at 4 ºC (refrigerator) for 14 hours. Then samples were rinsed thrice (2 minutes for each rinse) with 0.1M sodium cacodylate buffer (pH 7.0). Post-fixation was done in 2% osmium tetroxide in cacodylate buffer for 2 hours. Again rinsed thrice in buffer with each rinse for 2 minutes. Then tissue samples were dehydrated in ascending grades of ethyl alcohol. Then critical point drying done with carbon dioxide, followed by cryofracturing and mounting in colloidal silver paste on the stub. Then the specimen was sputtered with gold palladium and

observed under VEGA TESCAN (SEM) in CARISM Centre of SASTRA University, Thanjavur.
The observations in both samples were limited to the white pulp at 4000 to 5000 magnification.

Results and Discussion
The observations made in the specimen prepared by conventional processing for SEM was illustrated as Figure 1, and the observations made in the deparaffinized specimen was illustrated as Figure 2. Epithelial reticular cells, T lymphocytes, B lymphocytes, plasma cells, dentritic cells and interdigitating cells were observed in both methods at SEM HV 3.0kV. The clarity and contrast of depth of focus was better at magnification of 4000 in deparaffinized specimen (Fig. 2). But similar clarity and contrast of focus was obtained only at 5000 magnification in conventional SEM preparation (Fig.1). The line of cleavage in conventional EM preparation was found to be random, while the line of fracture of the section was continuous, precisely oriented in the paraffin sections of known thickness where intracellular details could also be noted because of cuts on the cells at certain planes (Fig. 2).

The paraffin processing method was simple and less expensive. This method offers several advantages over standard techniques presently used for SEM and TEM. Appropriate sections may be verified under light microscope before the effort is expended in preparing the specimen for SEM. Information available from light microscope may be directly correlated with the higher resolution obtained with SEM [7]. Handling the orientation of the section provides great flexibility in viewing the same subject from different perspectives. This flexibility combined with high resolution permits detailed examination of ultra structures [6]. The conventional EM processing sometimes involve impregnation of specimen by epoxy resins for long time storage. The procedures for removing the polymerized plastic was complex and often cause severe distortion of the specimen [9]. Cryofracturing of the specimen for conventional EM processing required specialized low temperature sectioning and critical drying equipment [5]. Further the line of fracture cannot be controlled, a single random line of cleavage would occur instead of the continous, precisely oriented serial sections of known thickness that are obtained by sectioning the paraffin embedded tissue.

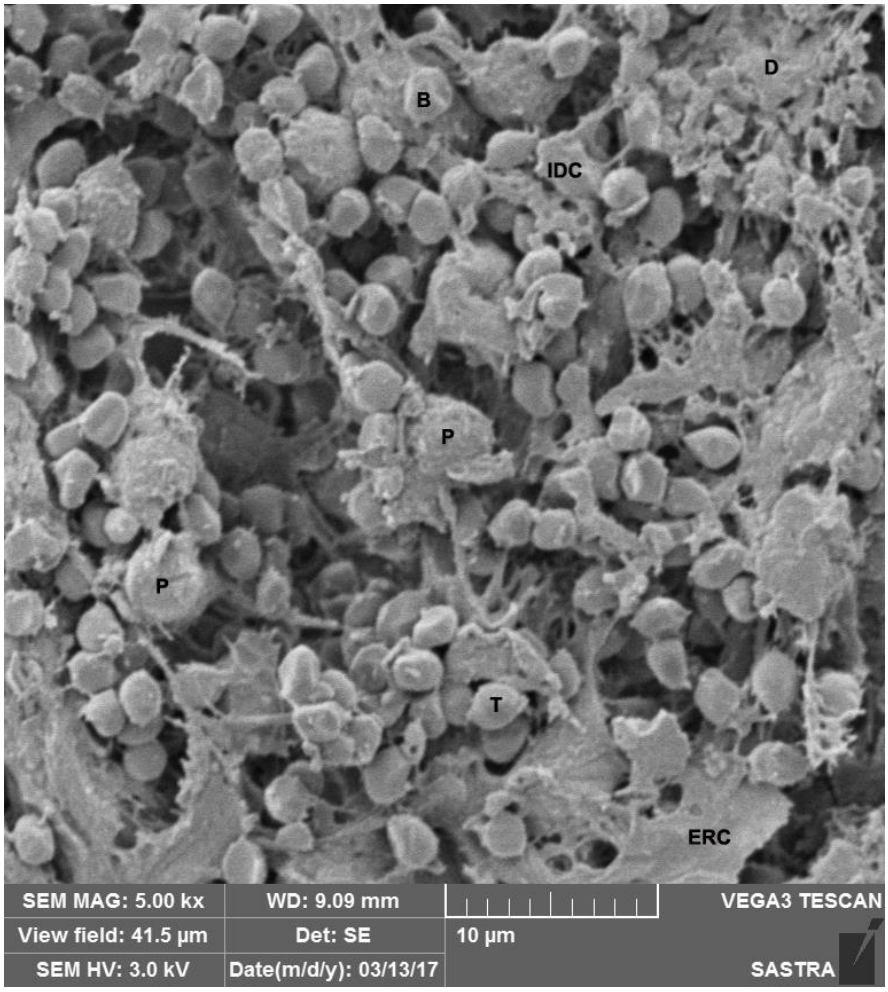

Fig 1: Scanning electron micrograph of the white pulp of spleen in 2 year old goat processed by conventional EM method. T – T lymphocyte, B – B lymphocyte, P – plasma cell, D – Dentritic cell, IDC – Interdigitating cell and ERC – Epithelial reticular cell.

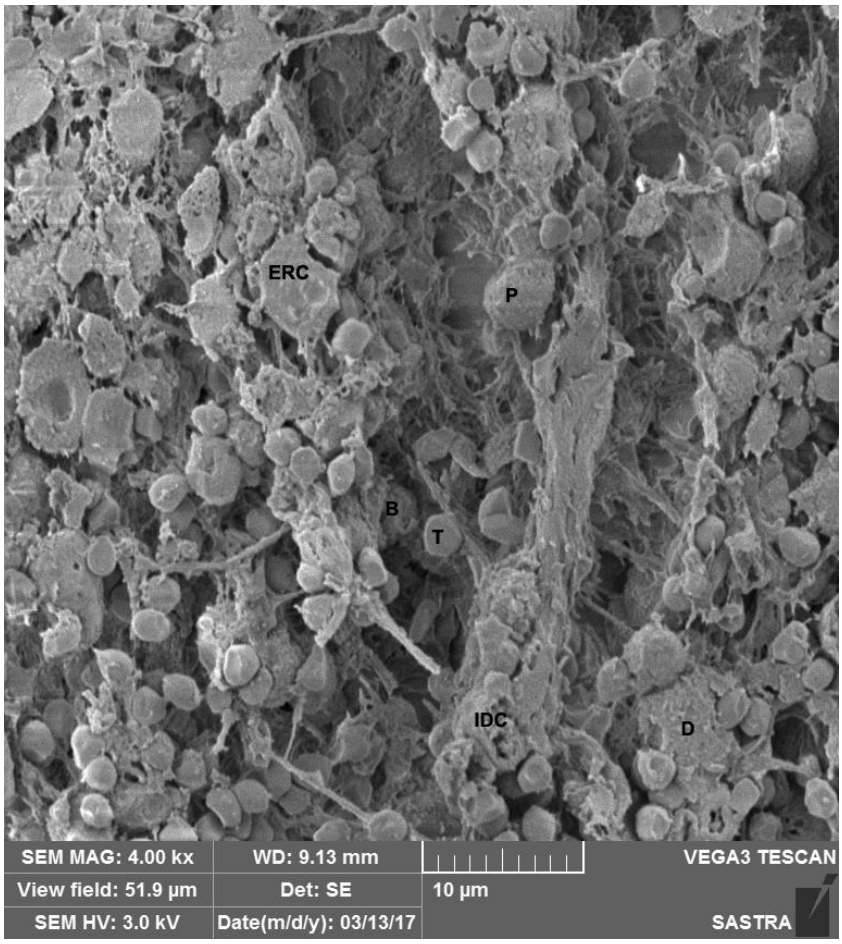

Fig 2: Scanning electron micrograph of the white pulp of spleen in 2 year old goat processed by Paraffin embedding method. T – T lymphocyte, B – B lymphocyte, P – plasma cell, D – Dentritic cell, IDC – Interdigitating cell and ERC – Epithelial reticular cell.

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