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RESEARCHARTICLE

A NOVEL TECHNIQUE OF PROCESSING TISSUES FOR SCANNING ELECTRON MICROSCOPY

Sivagnanam, S.

Department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University

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ABSTRACT

The conventional method of preparing specimens for scanning electron microscopy involves both glutaraldehyde and osmium tetroxide leading to considerable shrinkage of cells with appearance of artefacts. Present study eliminated the issues like shrinkage and occurrence of artefacts during SEM imaging.

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INTRODUCTION

Since the invention of electron microscopes the biological samples were processed by a Conventional method that required expensive chemicals and long time for processing the tissue to be observed under electron microscopes. The said conventional tissue processing involved glutaraldehyde, osmium tetroxide, uranyl acetate and lead citrate. The chemicals involved in conventional method tend to convert the biological sample into a metallic ultrastructure. Chances of appearance of artefacts in conventional processing is more.

The new technique is simple and involves inexpensive chemicals and short time to process. The images observed are devoid of artefacts. Hence the new technique is affordable and user friendly for processing the tissues for scanning electron microscopy.

MATERIALS AND METHODS

Tissue pieces (3mm cube) from spleen, lymphnode and thymus of goat immediately after slaughter was collected in Neutral buffered formaldehyde (NBF). The samples were fixed for 12 hours followed by overnight water washing. Then the samples were subjected to liquid dehydration by treating with 50% Isopropyl alcohol two changes each of one hour. Then samples were transferred to 70% Isopropyl alcohol two changes each of one hour. Then samples were transferred to 90% Isopropyl alcohol two changes each of one hour.

Then samples were transferred to 100% Isopropyl alcohol two changes each of one hour. By eighth hour sample is ready to be treated with xylene three changes each of 30 minutes. Samples were then transferred to hot air oven at 60°C overnight to accomplish evaporation of xylene from the tissue. Thereby the tissue was prepared in a single day and was ready to be sputter coated with gold or copper nano particles and subjected to Vega Tescan (Scanning electron microscope) between 8000 and 30,000 magnification.

RESULT AND DISCUSSION

Scanning electron microscopy of the tissue surfaces prepared by the current novel technique revealed good images of the cells and intercellular components without artefacts and shrinkage. Artefacts appeared in conventional method because of the heavy metals used during processing. Lead and uranium stains caused artefacts during imaging by Scanning electron microscopy (Thaete, 1979). The novel method did not use heavy metals for tissue processing resulting in complete absence of artefacts during imaging. Conventional method of drying for SEM, such as critical point drying, freeze–drying, as well as hexamethyldisilazane (HMDS)-drying caused shrinkage of cellular objects (Alisa *et al*., 2016). The sample processed by novel method had no shrinkage and may be subjected to freeze fracturing to demonstrate the deeper tissue architecture.

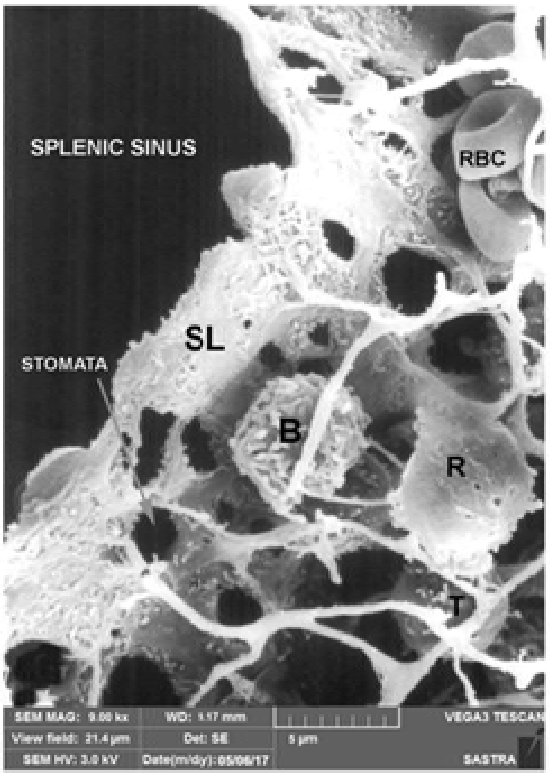

Figure 1 :SEM showing the red pulp of spleen in 8 to 12 months post-natal goat at 9000 magnification
SL - SINUS LINING CELL
B - B LYMPHOCYTE
T - T LYMPHOCYTE
R - RETICULAR CELL

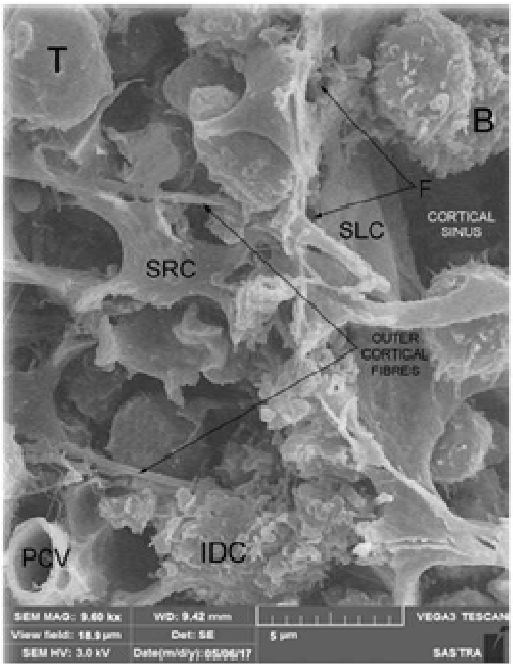

Figure 2 : SEM of outer cortex of mesenteric lymph node in 2 to 3 years old goat at 9600x magnification
T - T LYMPHOCYTE
B - B LYMPHOCYTE
SLC - SINUS LINING CELL
SRC - SINUS RETICULAR CELL
F - FENESTRATIONS
IDC - INTERDIGITATING CELL
PCV - POSTCAPILLARY VENULE

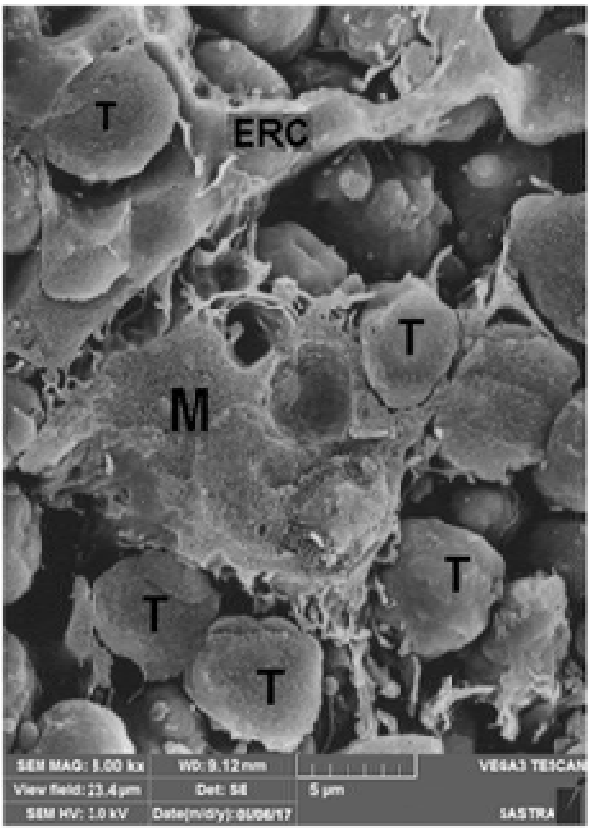

Figure 3 :SEM showing the cortex of thymus in 2 to 3 years old goat at 8000x magnification
M - MACROPHAGE
T - T LYMPHOCYTES
ERC - EPITHELIAL RETICULAR CELL

Unwanted fragments or tissue debris were removed during vacuum before sputtering with gold or copper nano particles. Every biological or medical scientist in the field of electron microscopy has to deal with the presence of troublesome artifacts caused by Conventional method of tissue processing for imaging (Varga *et al*., 2019). Present study revealed the structure of T lymphocytes, B lymphocytes, Dentritic cells, Interdigitating cells and macrophages in the spleen, lymphnode and thymus (Fig.1,2,3).

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