ORIGINAL ARTICLE

Received: December 2012
Accepted: February 2013

A study of the surface mucus changes overlying the transitional mucosa of the distal colon in malaysian patients

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ABSTRACT

Background: Colorectal cancer (CRC) which is the third most diagnosed cancer throughout the world is steadily rising in the Asian countries with the incidence rates mimicking the western counterparts. The pre-neoplastic nature of the Transitional Mucosa (TM) adjoining CRC lacks conclusive evidence so far. Pertaining to this, the study of the mucus layer, the functional component of the colonic mucosa overlying the TM is the subject of interest in this research.

Methods: The normal samples were biopsied from normal subjects by colonoscopy. Ten cases of CRCs of the distal colon who underwent anterior resection were chosen for this study. Three samples were taken 2ms, 5cms and 10cms from the colorectal cancer of the distal colon in the proximal margin. These samples were processed and viewed under the scanning electron microscope (SEM) to look for the changes in the mucus layer.

Results: The findings show that the changes were consistently found 2cms from the tumor and only one case showed changes up to 5cms. The statistical tests using SPSS version 18 revealed that there was no association between the surface mucus changes and the age, gender, race, site of tumor, differentiation of tumor and clinical staging of the patients.

Conclusion: It was concluded that there was no correlation between the structural and functional changes of the surface mucus overlying the distal colon during carcinogenesis. Furthermore, it can be stated that the functional change may be as a result of the structural change due to a nearby tumor.

Keywords: CRC, surface mucus, colonic mucosa, TM, scanning electron microscope.

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BCCR
2013; 5(1&2): 13-20
www.bccrjournal.com

Introduction

Colorectal cancer, in which the cells of the colon or rectum become abnormal and divide without control, is the third most commonly diagnosed cancer in males and the second in females worldwide with an incidence rate with over 1.2 million cancer cases and 608,700 deaths estimated to have occurred in 2008.[1] Colorectal cancer in Malaysia has steadily risen over the years and was the second most common cancer after breast in both the sex groups. It is ranked the first among males and second among females in Peninsular Malaysia.[2] The term Transitional mucosa (TM) came into vogue as early as 1969, when Filipe and her colleagues coined the normal looking adjoining mucosa of the colorectal carcinoma with abnormal secretions mucin secretions as TM.[3] Histochemical studies primarily using High Iron Diamine- Alcian Blue (HID-AB) stain show an increase in sialomucin production (blue/purple color) as compared to the sulphomucin (black/brown color) production in the normal colon.[4] Mucus in the large intestine secreted by the goblet cells protects the intestinal wall against excoriation which can be produced by the greater amount of bacterial activity as well as acidic nature of the feces apart from providing an adherent medium for holding the fecal matter.[5] The normal colonic mucus which is a mixture of both sulphomucins and sialomucins may vary in their proportions where the upper half of the crypt stains for both sulpho and sialomucins whereas the deeper half of the crypt is dominated by sulphomucins, characteristically found in the left colon.[4, 6-7] The presence of abnormal mucus at the resection margin may be a potentially important prognostic factor independent of the site, stage or differentiation of the tumor.[8] Study of the TM provides two important pieces of information for the clinician. First, the histochemical identification of the TM can be correlated with the tumor recurrences and patient survival. Second, patients with a higher risk for recurrence could be closely screened to increase the chance of complete cure.[9] Hence it is essential for a stringent follow-up of patients with significant transitional changes at the resection margins for a better treatment strategy to increase the chance of complete cure. [9, 10]Researchers have confined their observations on TM to mucin changes, commenting very little on the morphological features of the mucus overlying the typical TM.[11] The earliest changes would be in the surface mucus as it precedes that of CEA expression in the tissues as suggested by Mori and colleagues.[12] The Scanning Electron Microscope (SEM) which has the best resolution, wide range of magnification and great depth of focus which can be used to study the surface changes of the colonic mucosal layer.6 Hence, this research was undertaken to study the morphological changes of the surface mucus overlying the TM adjacent to the colorectal cancer of the distal colon in Malaysian patients as CRC stands the second in the overall incidence of cancers in Malaysia at present.2

Materials and Methods

The research was registered with the National Medical Research Register (NMRR id: 3027). Malaysia is composed of predominantly 3 ethnic races namely Malay, Indian and Chinese. The race was taken as a parameter for this study. The histopathology and differentiation of the tumor of the tumor was reported by the Department of Pathology HTAA, Kuantan, Malaysia. Patients who met the inclusion criteria were explained verbally and in written form on the objectives, procedures and risk of the study, before being asked for consent.

Normal colonoscopy samples

The normal colon samples were collected from patients undergoing normal colonoscopic examination in Dept of Surgery, Tengku Ampuan Afzan Hospital (HTAA) Kuantan. Subjects with no colon cancer or family history of colon cancer were selected for the study. The samples were taken using standard colonoscopy spikes (Brand; Radial Jaw 3). The samples were immediately fixed in the Mc Dowell fixative in labeled bijoux bottles to preserve the mucus layer. The bottles were then chilled in the ice box.

Samples from colorectal cancer resected specimens

Samples were taken from ten patients following anterior resection for tumor of the distal colon (descending colon/ sigmoid colon/ rectum). Three sites were selected i.e. 2cms, 5cms and 10cms from the tumor border in the proximal resected margin as shown in Figure 1. Three sites were selected because TM is prone to skip lesions.

Samples of size 1 cm2 were removed from the above three sites and the samples were immediately fixed in the Mc Dowell fixative in labeled bijoux bottles. The bottles were then chilled in the ice box.

Methodology

The processing of the samples was carried out in the Electron Microscopy Unit of International Islamic University campus in Indera Mahkota, Kuantan. The samples were first washed with 0.1M Sodium phosphate buffer thrice, followed by post-fixation with Osmium Tetraoxide at 4°C for 2 hours. Then the samples were washed with 0.1M Sodium Phosphate buffer twice for followed by washing with double distilled water thrice.

Samples were then dehydrated through graded alcohol series, 50%, 75%, 95% and 100% followed by Acetone. The samples thus dehydrated were transferred into the Critical Point Drying specimen holder submerged in Acetone. After critical point drying, the samples were mounted onto a SEM specimen stub with a double-sided sticky tape. The stub was then put into the Gold Coating Machine and samples were coated with gold. Finally, the samples were viewed under the Zeiss Evo 50 Scanning Electron Microscope.

Results

Normal mucus layer

The normal mucus layer is homogenous and completely covers the underlying epithelium as seen in low power (**Figure 1**). It is because of this layer that the fine details of the underlying cells are obscured. The mucus adheres to the tips of the microvilli giving them a clubby appearance. In high power, this layer has fine fenestrations which are usually very tiny and hence do not expose the underlying epithelium (**Figures 2 and Figure 3**).

Mucus layer overlying the TM at 2cms, 5cms and 10cms from the tumor

The morphological changes of the mucus layer overlying the transitional mucosa was studied at three levels from the tumor in the proximal margin. Out of 10 cases, all the cases showed significant mucus changes at 2cms. Only one case showed changes at 5cms. All the cases had a normal mucus layer at 10cms. The morphological changes of the mucus layer included the loss of layer form and formation of clumps of mucus on the surface, exposing the underlying mucosal surface as shown in **figures 5**. The changes in the mucus layer were identified as the increase in the size and number of fenestrations (**Figure 6**). These fenestrations continued to enlarge and coalesced to cause discontinuity in the mucus layer. This produced a cobweb appearance of the mucus (**Figure 7**).

Statistical analysis

All the results were analyzed by the Fischer Exact test, Krsukal-Wallis test and Chi-square test using the version 18 of Statistical Package for Social Sciences (SPSS). The values were considered significant if $p<0.05$ was obtained.

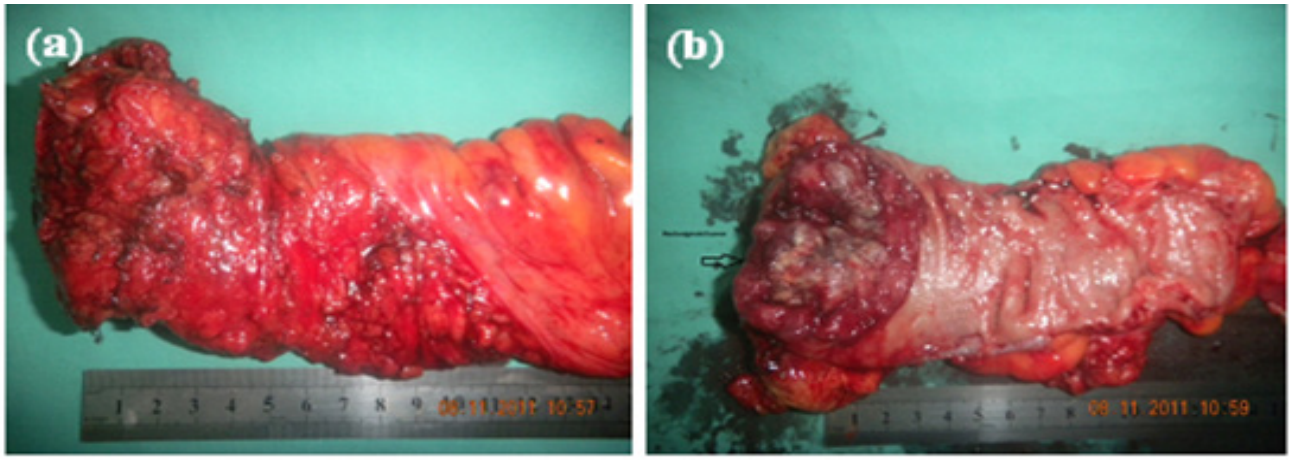

Figure 1: (a) Image showing a sample of a sigmoid cancer in a 60 year old female Malay patient. (b) Image showing the opened and cleaned out sample from the same patient.

Table 2: Table showing the grading of the tumors			
S.No	**TNM staging**	**Duke's staging**	**MAC staging**
1.	T3N1M0	C	C2
2.	T3N0M0	A	B1
3.	T3N2M0	C	C2
4.	T3N2M0	C	C2
5.	T3N0M0	C	C2
6.	T3N1M0	A	B1
7.	T4N2M1	C	C1C2
8.	T3N0M0	B	B2
9.	T4N1M1	C	C2
10.	T2N1M0	C	C1
TNM- tumor, node, metastasis			

Table 3: Table showing the particulars of the patients taken up for the study							
S. No	**Age in yrs**	**Sex**	**Race**	**Type of cancer**	**Clinical staging**	**Site of tumor**	**Differentiation of tumor**
1.	60	F	Malay	Adeno CA	III	Sigmoid	Moderate
2.	23	F	Malay	Adeno CA	I	Sigmoid	Well
3.	58	M	Malay	Adeno CA	III	Mid rectum	Moderate
4.	61	F	Malay	Adeno CA	III	Low rectum	Moderate
5.	63	M	Malay	Adeno CA	III	Low rectum	Moderate
6.	59	M	Malay	Adeno CA	I	Mid rectum	Moderate
7.	35	F	Non-Malay	Adeno CA	III	Mid rectum	Moderate
8.	67	M	Malay	Adeno CA	II	Sigmoid	Moderate
9.	74	F	Malay	Adeno CA	III	Low rectum	Moderate
10.	74	F	Malay	Adeno CA	III	Low rectum	Moderate
Yrs –years, M-male, F-female, CA-carcinoma							

Discussion

The functions of the mucus layer are closely related to the structure of the mucus layer and any alteration of the chemical composition may alter its protective abilities.[13]

Any diseased condition of the intestinal glands will lead to a change in the composition and integrity of this mucus layer. This change exhibits as morphological alterations which are well appreciated by the scanning electron microscope. Studies in the chemical carcinogenesis of rats as well as on human colorectal cancer lesions have

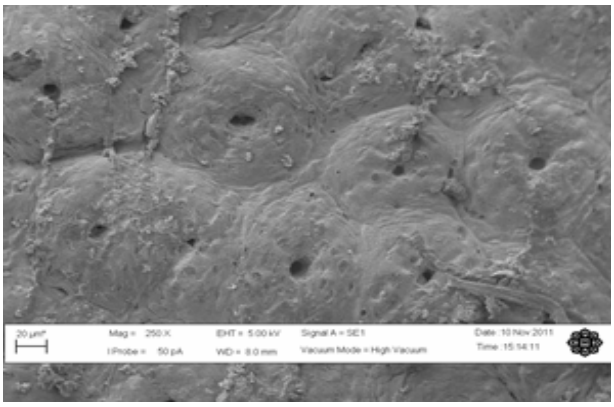

Figure 2: SEM image 250 X showing the surface of the colon covered by mucus obscuring the fine detail of the crypts and the cells.

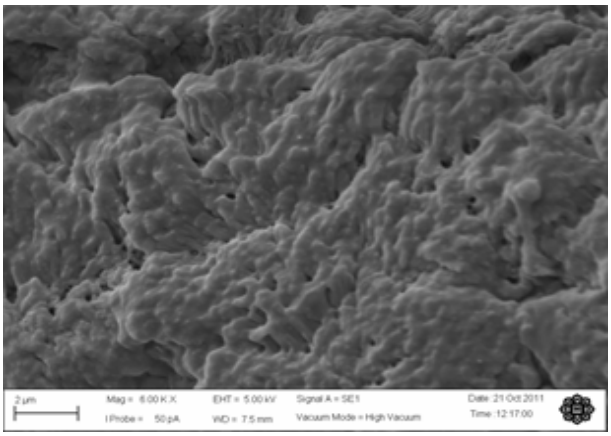

Figure 4: A high power image 6000 X showing tiny fenestrations.

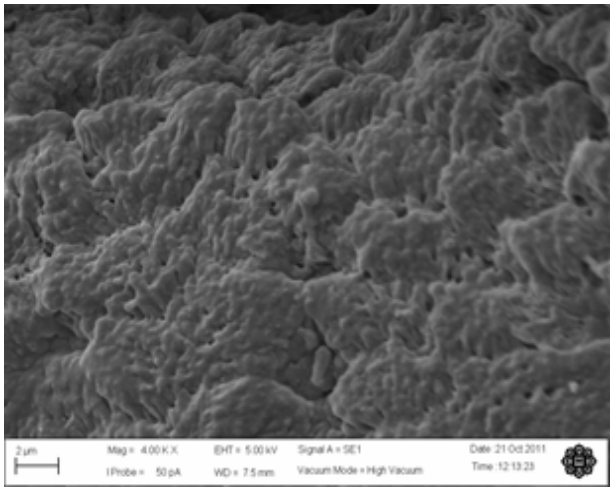

Figure 3: SEM image of high power 4000 X, showing the normal mucus layer.

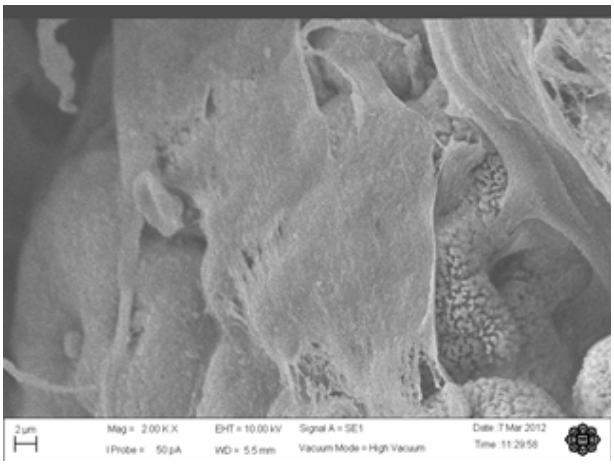

Figure 5: High power SEM image 2000 X at 2cms showing the tearing of the mucus layer to expose the underlying mucosal layer in a 67 yr old male patient with sigmoid cancer.

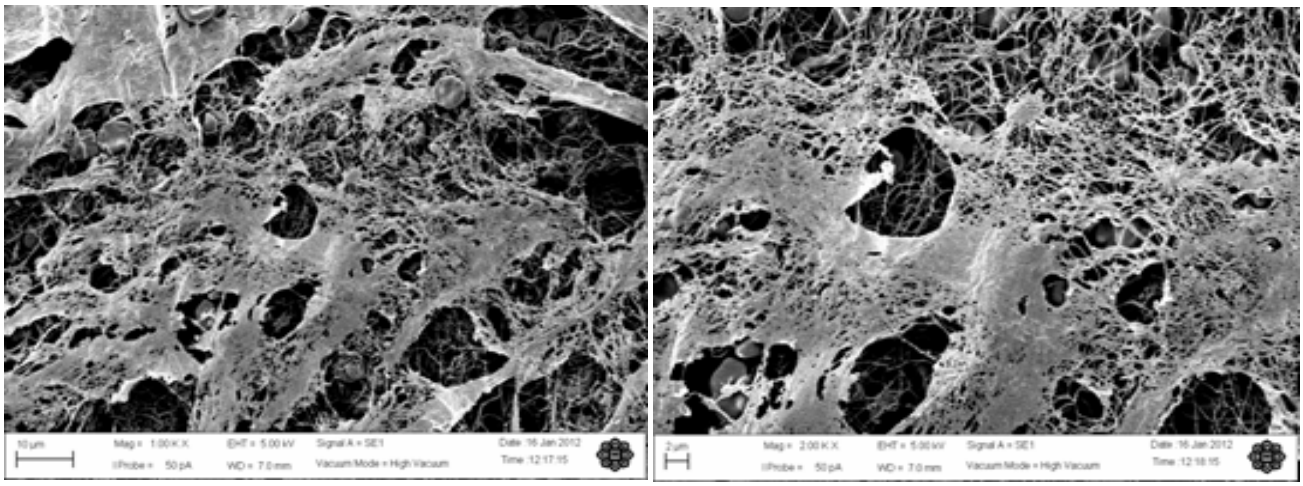

Figure 6: SEM images 1000 X & 2000 X showing the cobweb appearance of the disintegrated mucus layer seen 2cms from the tumor in a 61 yr old female patient with rectal cancer.

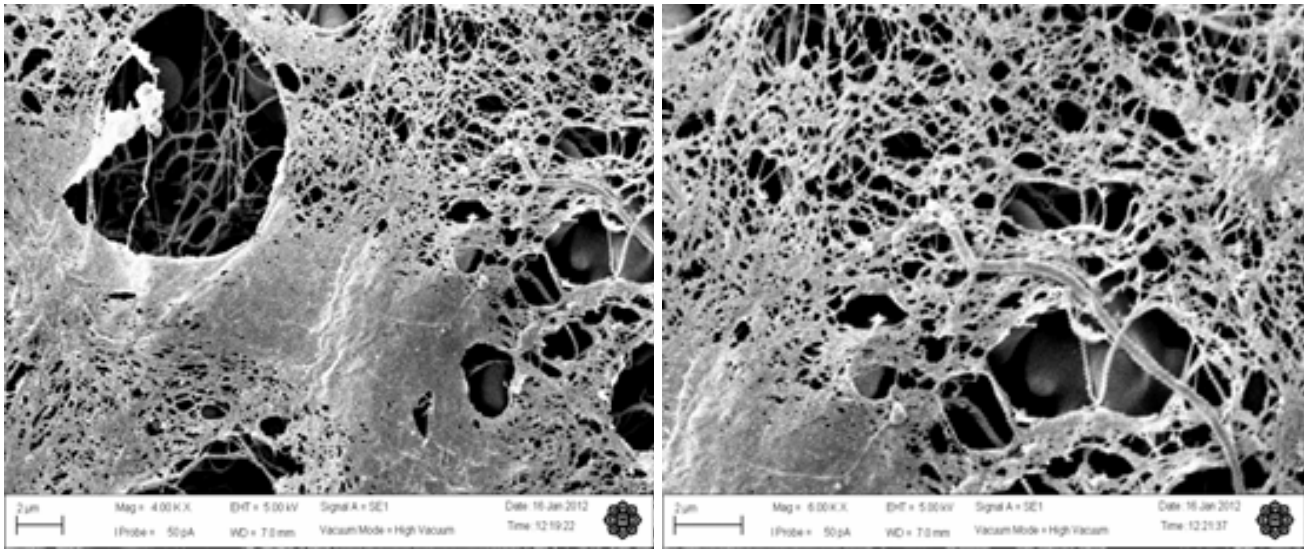

Figure 7: High power images 4000 X & 6000 X of the mucus layer.

Table 3: Table showing the association of the age, gender, race, site, differentiation and clinical staging of tumors with the surface mucus changes in the 10 cases studied.

Variables		Mucus changes				Statistical analysis	df	P* value
		Only At 2cms Mild		Only At 2, 5cms Severe				
		No	%	No	%			
Age (yrs) Median (IQR)	**60.5 (17)**	3	30%	7	70%	Kruskal- Wallis test	1	.493
Gender	**Male**	1	10%	3	10%	Fisher exact test	1	.600
	Female	2	20%	4	40%			
Race	**Malay**	3	30%	6	60%	Fisher exact test	1	.900
	Non Malay	0	0	1	10%			
Clinical staging	I	1	10%	1	10%	Chi square test	2	.788
	II	0	0	1	10%			
	III	2	20%	5	50%			
Differentiation of tumor	Well	2	20%	7	70%	Fisher exact test	1	.900
	Moderate	1	10%	0	0			
Site of tumor	Sigmoid	1	10%	2	20%	Fisher exact test	1	.700
	Rectum	2	20%	5	50%			

*P value significant <0.05, Yrs- years, IQR-Inter quartile range, No- number of cases, df- degree of differentiation

proved progressive disintegration of this layer. [9, 14] An association between the changes of this layer and the epithelial surface changes can give us a clue to the severity of the changes of the transitional mucosa and thence give

us a correlation between the structural change and the functional change.

In this study the surface mucus layer was studied using the SEM. This layer was studied at three levels i.e 2cms, 5cms and 10cms from the tumor in the proximal resected margin. Care was taken to preserve the mucus layer by minimal handling of the tissue, not washing the tissue with a jet of saline and not cleansing the tissue. The changes noted in the mucus layer were consistently found at about 2cms from the tumor margin in all the ten cases with only one case of stage III low rectal tumor showing the changes at 5cms from the tumor. So the results suggest that the 90% of patients exhibit mild mucus changes only. However, there was no association between age, gender, race, site of tumor, differentiation of tumor and clinical staging with that of the surface mucus changes.

The changes of the mucus layer can be either primary, exposing the underlying epithelium to the carcinogens or secondary to the cancer of the mucus secreting cells. This good but inconclusive evidence can give a clue to the mechanism of carcinogenesis. The alterations in the histochemical properties are due to the predominance of the sialic acid moiety. Sialic acid is the component which determines the visco-elastic properties of the mucus and this substantiates the loss of the mucus layer overlying the transitional mucosa in this study.[14] The changes are due to the alteration in the rheological properties of the mucus as sialic acid is the key factor in the determination of this property which leads to loss of cohesion and the breakdown of the mucus into clumps without retaining their layer form.[13] Another possible explanation for the loss of mucus layer can be attributed to the inevitable reduction in the amount of mucus secretion by the reduced goblet cells, a feature commonly encountered in the early stages of carcinogenesis. This results in the thinning of the mucus layer over the mucosal surface.[13]

Since the changes were predominantly found at 2cms from the tumor margin, it can be suggested that the surface mucus changes were due to the direct continuation of TM from the tumor similar to Wang and colleagues.[10]

Conclusion

The present study is an attempt to characterize the SEM findings of the of the mucus layer overlying the TM adjoining the colorectal carcinoma and correlate the structural and functional changes. In this study, ten patients expressed mild surface changes predominantly (90%) similar to the previous studies involving the morphology of the surface mucus changes which have been confined to 2cms from the tumor14. Only one patient (10%) showed changes at 5cms from the tumor margin. There were no changes noted at 10cms in all the ten patients. The statistical tests proved that there was no association between the surface mucus changes and the age, gender, race, site of tumor, differentiation of tumor and clinical staging of the patients. Hence it can be concluded that there is no correlation between the structural and functional changes of the surface mucus overlying the distal colon during carcinogenesis. Furthermore, it can be stated that the functional change may be as a result of the structural change due to a nearby tumor. As a further study, an association between the changes of this layer and the epithelial surface changes can give us a clue to the severity of the changes of the transitional mucosa and thence give us a correlation between the structural change and the functional change. It can also be hypothesized that the functional changes might follow the structural changes but this still lacks evidence. A study involving a larger group of patients might be rewarding in this regard.

Acknowledgements

The work was carried out in Hospital Tuanku Ampuan Afzan (HTAA), Kuantan, Malaysia and International Islamic University (IIUM) Kuantan, Malaysia. This study was funded by the IIUM Research Endowment Fund Type B (Grant no: EDWB 0803-110). The support rendered by the surgeons and the staff of the Department of Surgery, IIUM and HTAA as well as the technicians of the Electron Microscopy Unit, IIUM is greatly acknowledged.

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