UNIVERSITI SAINS MALAYSIA

PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA PENDEK

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan **dua (2) salinan** laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/ Pengarah/ Ketua Jabatan kepada Pejabat Pengurusan dan Kreativiti Penyelidikan (RCMO)

1. **Nama Ketua Penyelidik:** Dr Badrul Hisham Yahaya
   Name of Research Leader

   ☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):** Advanced Medical and Dental Institute (AMDI)
   School/Department

3. **Nama Penyelidik Bersama:** -
   Name of Co-Researcher

4. **Tajuk Projek:**
   Title of Project Analysis of Pathophysiological Changes of the Airway Epithelium During Regeneration and Repair Following Tracheal Brushing in Rabbit

5. **Ringkasan Penilaian**/*Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☑	☐
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☑	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☑	☐	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☑	☐	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☑	☐	☐	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☑	☐

6. Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English). This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Understanding the mechanisms during airway repair is essential to develop a therapeutic approach towards lung-related diseases. The airway epithelial layer undergoes well-defined repair stages such as of cell migration, proliferation, and redifferentiation in response to injury. In order to study the cellular mechanisms respond to injury and repair, we imposed an injury on rabbit's trachea airway epithelial layer by performing brushing technique. This technique has advantage over the previous technique which not requires surgical approach, time-efficient and less risk of infection. After performed the tracheal brushing, rabbit were euthanized at different time points - 30 min, 1 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72hr , 96 hr,7 day, 14 day, and 21 day (n=3 for each time point including naïve control animals). The length of the induced-injuries were measured between the edges of the remaining epithelium bordering the lesion and the results found that the length of the injured areas were gradually decreased over the time points as compared to 30 min following injury. The decreases of the length of the injuries indicate that regeneration process was activated as to restore the normal epithelial layer. Various histopathological responses were observed from the 30 min to 21 day post brushing, from completely removed of the epithelium layer and its basement membrane until some of injured areas were covered by series of single cells layer at 21 day. We had successfully developed a more practical and time-efficient brushing techniques with less infection risks and thus could be very useful technique in order to study cellular and molecular mechanisms during airway regeneration and repair which is comparable to the study carried out in larger animal model such sheep and calve.

Pemahaman berkenaan dengan mekanisma pembaikpulihan epithelia saluran pernafasan adalah penting ke arah menghasilkan satu teraputik penyakit yang berkait dengan peparu. Epithelia Saluran pernafasan yang tercedera akan melalui beberapa proses pembaikpulihan termasuk migrasi sel, proliferatif sel, dan redifferentiation sel. Didalam memahami proses ini, epithelia saluran pernafasan haruslah diberikan kecederaan terlebih dahulu dengan menggunakan teknik pemberusan. Teknik ini mempunyai kelebihan berbanding teknik-teknik lain yang pernah digunakan sebelum ini. Antara kelebihan tersebut termasuklah, tidak menggunakan sebarang teknik surgeri, pantas, dan kurang terdedah kepada kemungkinan bahaya jangkitan. Selepas melakukan teknik pemberusan pada trakea, arnab seterusnya dibunuh selepas mencapai titik masa berikut: 30 min, 1 jam, 6 jam, 12 jam, 24 jam, 48 jam, 72 jam, 96 jam, 7 hari, 14 hari, dan 21 hari (jumlah arnab pada tiap titik masa termasuk normal = 3 ekor). Panjang kawasan "penyingkiran" diukur diantara dua titik hujung tinggalan epithelia saluran pernafasan. Keputusan menunjukkan panjang kawasan ini menurun bermula pada titk masa 30 min dan seterusnya merentasi titik masa yang lain. Pola penurunan ini menunjukkan proses pembaikpulihan telah diaktifkan. Pelbagai perubahan histopatologi dapat dilihat pada titik masa 30 min sehingga 21 dari. Perubahan ini bermula dengan penyingkiran lapisan epithelia saluran pernafasan sehingga penurapan satu lapisan sel pada kawasan tersebut semasa hari ke-21. Kami telah berjaya membangunkan teknik pemberusan yang praktikal, pantas, dan kurang terdedah kepada kemungkinan bahaya jangkitan. Teknik ini sangat berguna dalam mengkaji penglibatan sel dan molekul semasa proses regenerasi dan pembaikpulihan. Keputusan yang didapati ini setanding dengan penemuan kajian yang dilakukan pada kambing biri-biri dan anak lembu.

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	**Bahasa Inggeris**
Teknik pemberusan	Brushing technique
Epitelium saluran pernafasan	Airway epithelium
Kecederaan teraruh	Induced-injury
Gerak balas sel	Cellular changes

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

Three publications:

1.	Type: Research article Title: Blinded Brushing Technique as a Novel Method to Inflict Injury on Rabbit Tracheal Airway Epithelium Structure Author: Ahmad Zaeri Latahir and Badrul Hisham Yahaya Publication year: 2012 Journal: Journal of Animal and Veterinary Advances Impact factor: 0.392
2.	Type: Review article Title: The Rabbit as a Model for Studying Lung Disease and Stem Cell Therapy Author: Nurfatin Asyikhin Kamaruzaman, Egi Kardia, Nurulain 'Atikah Kamaldin, Ahmad Zaeri Latahir, Badrul Hisham Yahaya Publication year: 2013 Journal: Biomed Research International Impact factor: 2.542
3.	Type: Journal Proceeding Title: Analysis of Pathophysiological Changes in Regeneration and Repair of Rabbit Airway Tracheal Epithelium Response to Induced Injury Publication year: 2012 Author: Ahmad Zaeri Latahir and Badrul Hisham Yahaya Journal: Regenerative Research Journal-Electronic E-ISSN 2232-0822

Two Conferences:

1.	Name of conference: 4th Malaysian Tissue Engineering and Regenerative Medicine (MTERMS) Scientific Meeting Type: Poster Presentation Title: Analysis of Pathophysiological Changes in Regeneration and Repair of Rabbit Airway Tracheal Epithelium Response to Induced Injury Author: Ahmad Zaeri Latahir and Badrul Hisham Yahaya Date: 3-4 June 2012 Place: Meritus Pelangi Beach Resort & Spa,Langkawi, Malaysia * Published in Regenerative Research Journal-Electronic E-ISSN 2232-0822
2.	Name of conference: 17th National Conference on Medical and Health Sciences Type: Poster Presentation Title: Blinded Brushing Technique: A Novel Method Inflict Injury on Rabbit Tracheal Airway Epithelium Author: Ahmad Zaeri Latahir and Badrul Hisham Yahaya Date: 27-28 May 2012 Place: Universiti Sains Malaysia, Health Campus, Kelantan.

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

One postgraduate student:

Name: Ahmad Zaeri Latahir
Degrees: Master of science (Genetic)
Status: Active
Institution: Advanced Medical and Dental Institute (AMDI), USM

ii) Lain-lain:
Others

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

Tandatangan Penyelidik
Signature of Researcher

18/3/13

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Tutup Geran.
Baki Geran ?

13/8/13

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

PROFESOR ABD AZIZ TAJUDDIN
Pengarah
Institut Perubatan dan Pergigian Termaju
Universiti Sains Malaysia

19/8/2013

Tarikh
Date

TECHNICAL REPORT OF SHORT-TERM GRANT

Dr Badrul Hisham Yahaya

Advanced Medical and Dental Institute (AMDI)

Universiti Sains Malaysia

Title:

Analysis of pathophysiological changes of the airway epithelium during regeneration and repair following tracheal brushing in rabbit

Introduction

The process of airway epithelial repair subsequent to severe injury appears to follow well-defined stages. Common features that seem to characterize airway epithelial repair following physical injury involve the dedifferentiation of cells bordering the lesion, migration of the flattened cells over the wound area, proliferation and re-differentiation. In particular, various cell types, including basal, ciliated and secretory cells, appear to possess the capacity to dedifferentiate, to migrate, and to either re-differentiate, or trans-differentiate to give rise to other types of cells. Indeed secretory cells have been shown to dedifferentiate and become flattened epithelial cells when seeded in normal tracheal epithelial cell culture and thereafter re-differentiate to normal morphology whilst basal cells were less frequently observed in a similar process [1]. These observations further suggest that potential may exist to manipulate conditions following injury or even in the context of airway disease such that the airway epithelium can be restored to its optimum.

Adult lung stem cells are capable of abundant self-renewal and regeneration, and should act as stem/progenitor cells in response to injury and effect local repair [2-6]. As such, these cells may serve as a viable target to manipulate this process in the context of the abnormal repair patterns that threaten normal lung physiology. Several cell types of the lung capable of functioning as stem/progenitor cells in response to injury have been identified; these cells are thought to localize to proximal airway sub mucosal gland ducts, intercartilagenous ring regions, neuroepithelial bodies, and terminal bronchioles/bronchoalveolar duct junctions [7-10]. The cells identified as progenitor or stem cells in the lung appear to vary according to

the lung compartment [3, 11-15]. Equally these observations potentially imply that the specific repair mechanisms evoked in response to lung injury will draw upon several sources of stem/progenitor cells according to the nature and extent of the damage. In this regard it has been suggested that slight or moderate injury will result in resident progenitor cell activation to restore tissue homeostasis whereas severe injury and extensive epithelial cell loss will promote stem-mediated repair [16].

However, due to very low rates of cellular proliferation *in vivo* in the normal steady state, and the complexity of cellular and architectural of the respiratory tract, lung stem cells remain poorly understood compared to those in other major organ systems. Therefore, this study is designed to understand the nature of cellular behaviour towards repair of the airway epithelium following injury and to investigate potential stem/progenitor cell types to be targeted as future cell-based therapy in lung-related disease. The use of rabbit as our animal model system to mimic the human condition is due to the fact that this animal has been reported a good model for experimental study in a model of ischemia-reperfusion lung injury during cardiopulmonary bypass [17], effects of acute nitrogen dioxide intoxication [18], as a model of lung parenchymal and tracheal injury [19] and to study a proliferation during early phases of bronchiolar repair in neonatal rabbits [20]. In fact, the use of tracheal brushing technique has been reported by Nakagishi (2005) in which the tracheal mucosa was scraped with a nylon brush in order to induce the airway stenosis [21]. However, a different strategy was used in our study: the brushing technique was developed with a simple laboratory setting without any advance equipment. By using this technique, it facilitates us to study on the pathological changes of airway epithelium at specific time point after infliction of injury. Due to budget constraint, only single brushing was adapted in this current study whilst repeated brushing was dropped (as proposed in the original proposal).

Objectives

1. To study the effect of brush-induced injury on the histopathological changes of the airways
2. To investigate the time course-effect of the histopathological changes of the airways in response to injury

Methodology

Thirty six New Zealand White rabbits were used in this experiment. The rabbits were grouped into untreated (normal), and brushed (based on different time points): 30 min, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 7 days, 14 days, and 21 days. Three rabbits were allocated for normal group and each of the time point for treated group. Rabbits were anaesthetised by administering intramuscular ketamine (35mg/kg) (Troy ilium, Australia) and xylazine (Troy ilium, Australia) (3mg/kg). Brushing technique was performed to induce injury into the tracheal epithelial airway using an interdental brush (Oral-B, US) with 5.5 mm diameter bristle was inserted into the mouth through an endotracheal tube (Figure 1 & 2). Euthanasia performs at 30 min, 1 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 7 days, 14 days, and 21 days after brushing. Normal rabbits were euthanized without any prior brushing.

The trachea tissues were trimmed and fixed in 10% formalin solution for 24 hours. The trachea was cut laterally into different section with approximately 0.5cm thick. The tissues were processed and individually embedded in paraffin wax and cross sectioning into 5µm thick using microtome (Lieca, Germany). The sections were subjected to standard haematoxylin and eosin (H&E) staining. The sections were viewed under light microscope (Olympus, US) and captured using image analyser software (Soft Imaging System Olympus, US). The present of injury was confirmed when the loss of the epithelial layer and/or its basement membrane were observed. Length of injury was measured between two edges of remaining epithelial layers bordering the lesion.

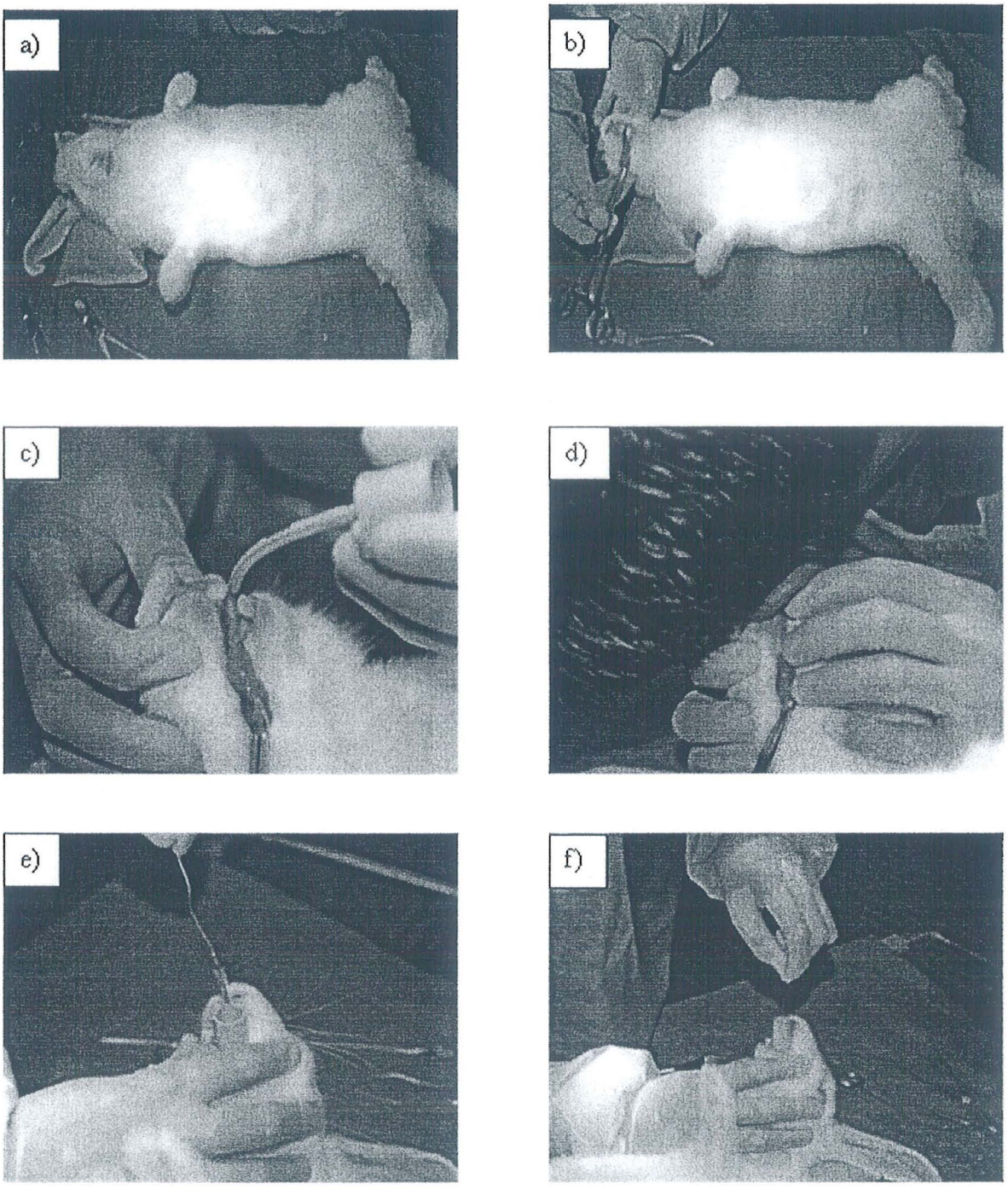


Figure 1: Sequential procedure of conducting brushing technique on rabbit's trachea. a) Anaesthetised rabbit was laid in supine position. b) The tongue was pulled aside to make the opening of the mouth broader. c) Endotracheal tube was inserted into the trachea. d) Listen to the breathing sound to confirm that the tube was in the trachea. e) The interdental brush attached to steel wire was inserted through endotracheal tube. f) Brushing was performed.

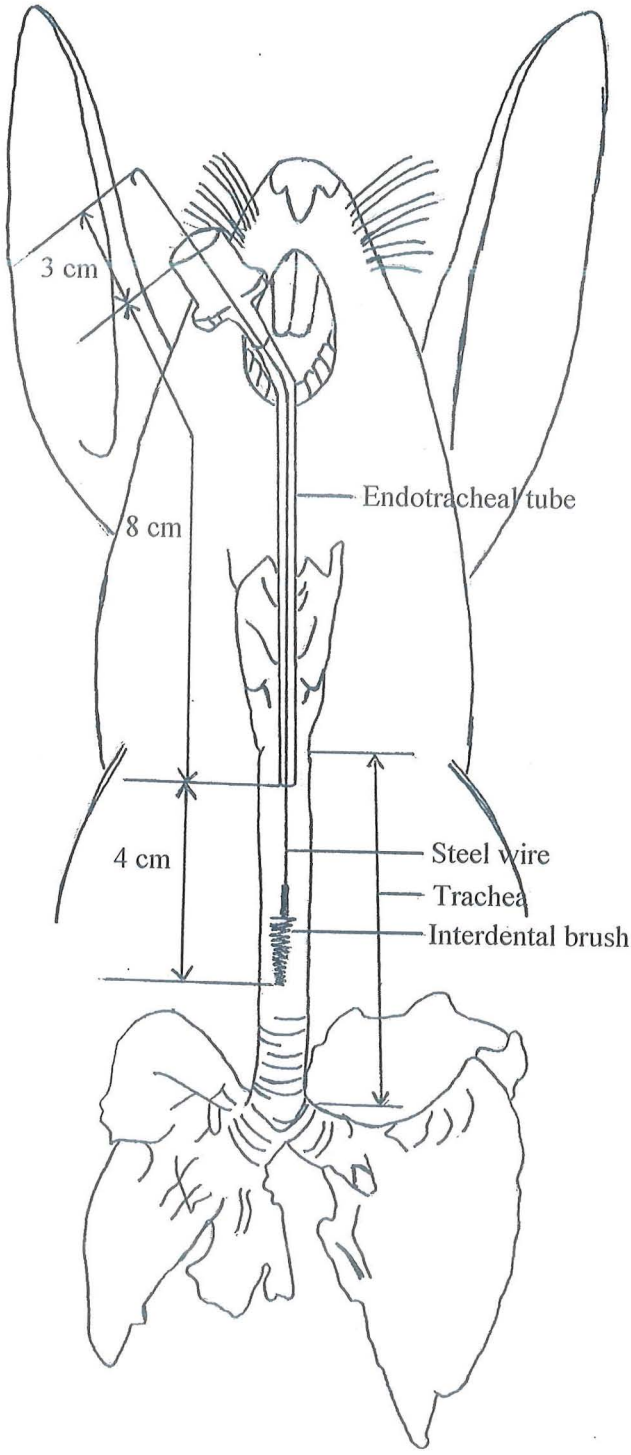
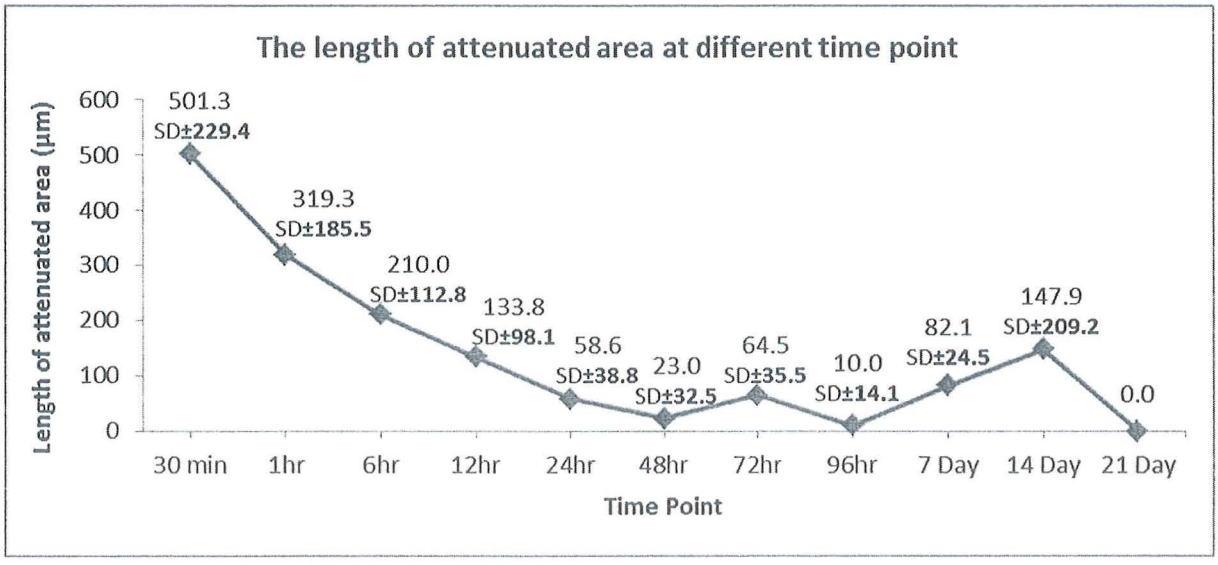


Figure 2: A schematic diagram of endotracheal tube and steel wire positioning in the rabbit tracheal airway. The area of epithelium brushed was approximately 4cm in length.

Result

The measurement of time-course effect of brushing on the length of the area of injury

The brushing has caused the loss of pseudostratified epithelium layer including the basement membrane. This has caused the submucosal region exposed to the lumen. The length of the injured/brushed area of every time point was measured and shown in graph (Graph 1). At 30 min until 48 hours post injury the length of injured area shows gradually shortened from 501.3µm to 23.0µm. The length of injured area has slightly increased at 72 hours and decreased at 96 hour. However, the length of injured area has increased sequentially on next two time points, 7 days and 14 days with the final length is 147.9µm. Finally, at day 21, there was no brushed area was left uncovered.



Graph 1: The length of injured areas at different time points. SD: standard deviation

Inflammatory response after brushed-induced injury

The inflammatory response is an essential response after injury. Disturbances of the normal physiological environment encourage infiltration of the cells and plasma into a tissue. At 30 minutes after injury, the early response to injury, submucosal area was populated by infiltrating neutrophils with a few eosinophils and basophils presence. Then at 1 hour, a few occasional infiltrated neutrophils presence in most of the submucosal area presented with the presence of lymphocyte. However, at 6, 12, and 24 post injury, very few inflammatory response seen across these time points. At 48 hours, neutrophils were populated at the area

infiltrated of plasma cell. At 72hours, mass lymphocyte in the submucosal area with the occasional presence of neutrophils. In addition, plasma cells were seen majorly presented in the area. After 96 hours, no clear presentation of inflammatory reaction was seen. At 7 days eosinophils were found scattered around the submucosal area. Whereas, at 14 days plasma cells and neutrophils were both present at this time point. None of the inflammatory response can be seen at 21 days.

Pathophysiological of the tracheal epithelium following brushing-induced injury at sequential time points

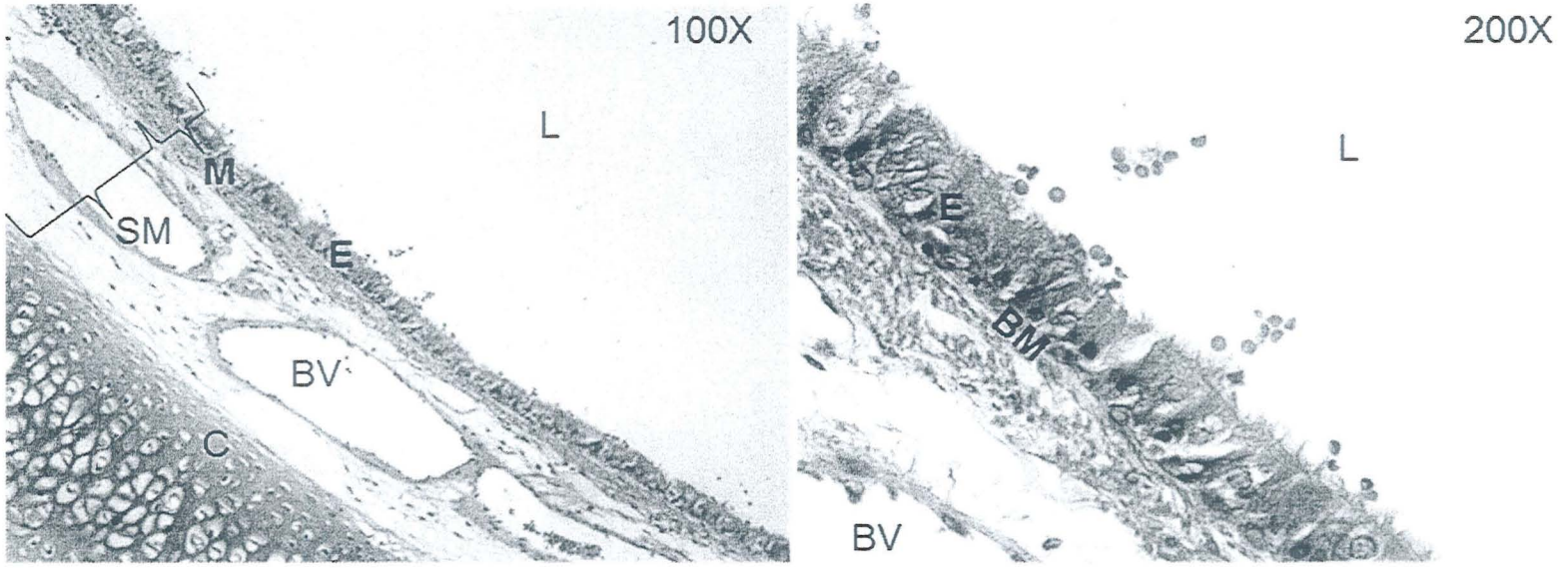


Figure 3. Normal trachea comprises of three layers, Mucosa (M), Submucosa (SM), and Cartilage (C) (100x). Cartilage situated at the lowest part of trachea which wrap around the whole layers that made up the trachea. Submucosa layer majorly populated with blood vessel located above cartilage. Mucosa layer as the uppermost layer of trachea consist of both basement membrane (BM) and pseudostratified epithelial layer (E) (200x). This epithelial layer facing to tracheal lumen (L).

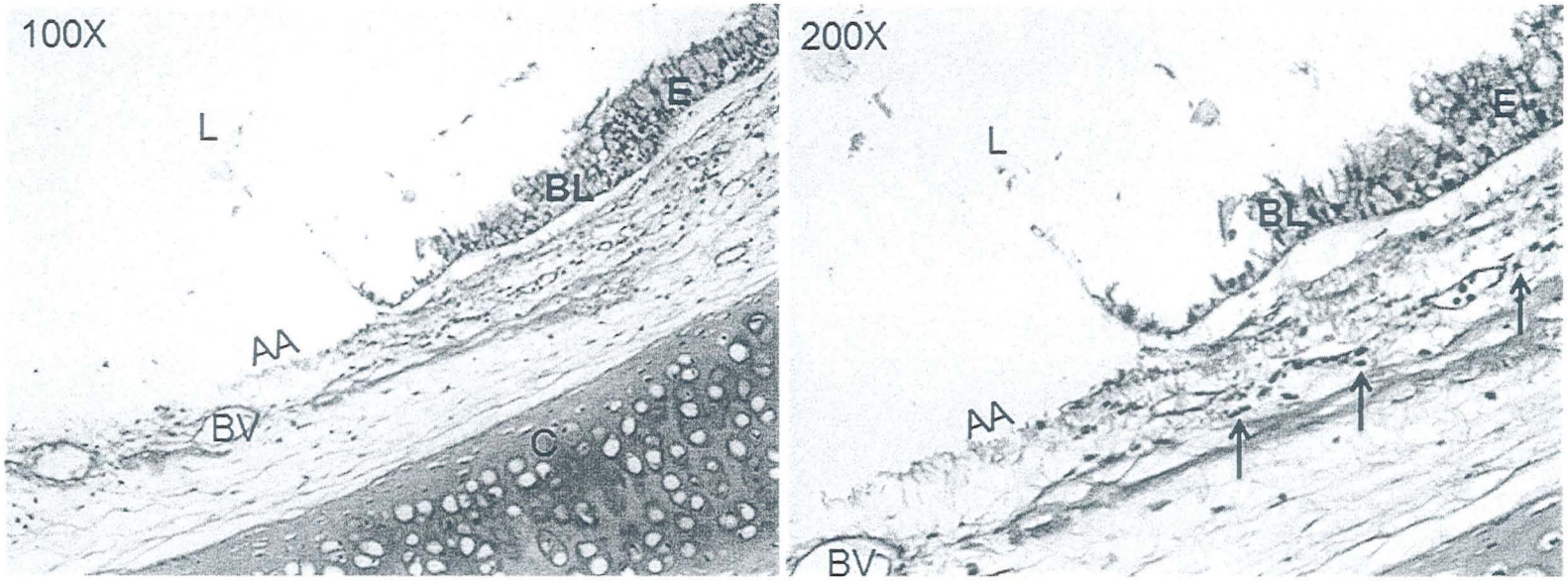


Figure 4. Thirty minutes post injury. At this time point, changes seen on epithelial layer due to the brushing impact. At this stage, Epithelial layer can be divided into three distinct areas. Attenuated area (AA) has complete loss of epithelium leaving the intact basement membrane. Bordering the lesion (BL) located between intact epithelium (E) and AA. This area has remained pseudostratified epithelium accompany with slight disruption in the apical area. Lymphocyte (arrow) seen scattered around the submucosa layer.

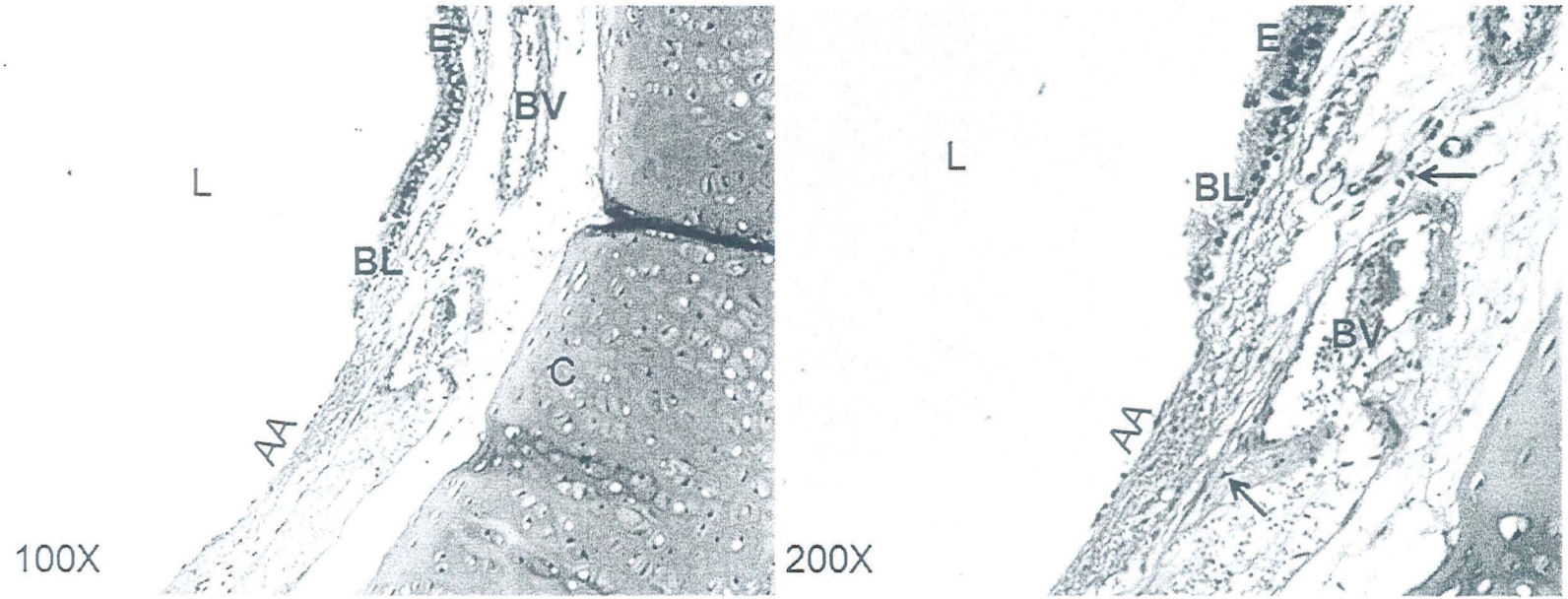


Figure 5. One hour post-injury. The changes of the epithelial layer are still observed on the three clear distinct areas of the epithelial layer (E), bordering the lesion (BL), and attenuated area (AA). The disruption clearly disorganised the structure of the epithelial layer. Occasional of lymphocyte scattered around the submucosa layer.

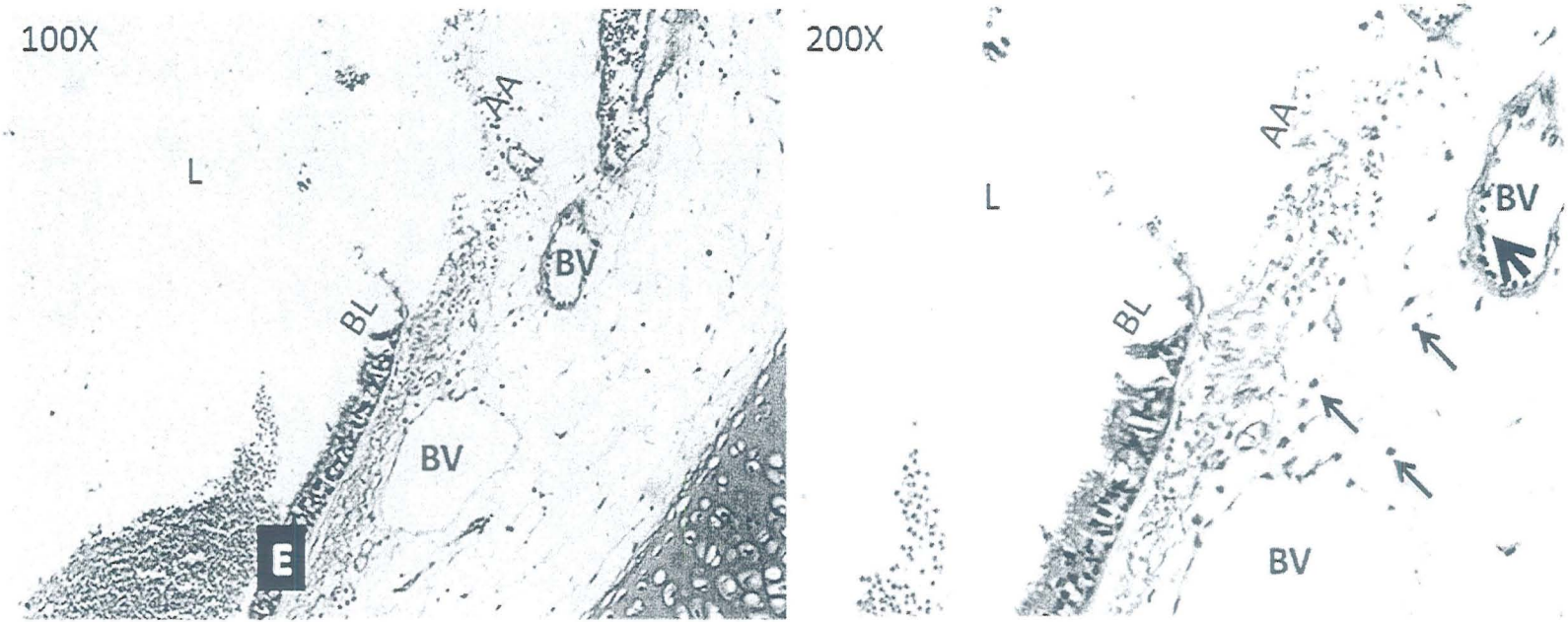


Figure 6. Six hours post injury. Brushing impact extensively damages the epithelial layer with damage causes disruption extend toward submucosa layer. The changes of the epithelial layer are still observed on the three clear distinct areas of the epithelial layer (E), bordering the lesion (BL), and attenuated area (AA). Occasional of lymphocyte surround the submucosa area (thin arrow). Lymphocyte is also perlocating the blood vessel (BV) wall (thick arrow).

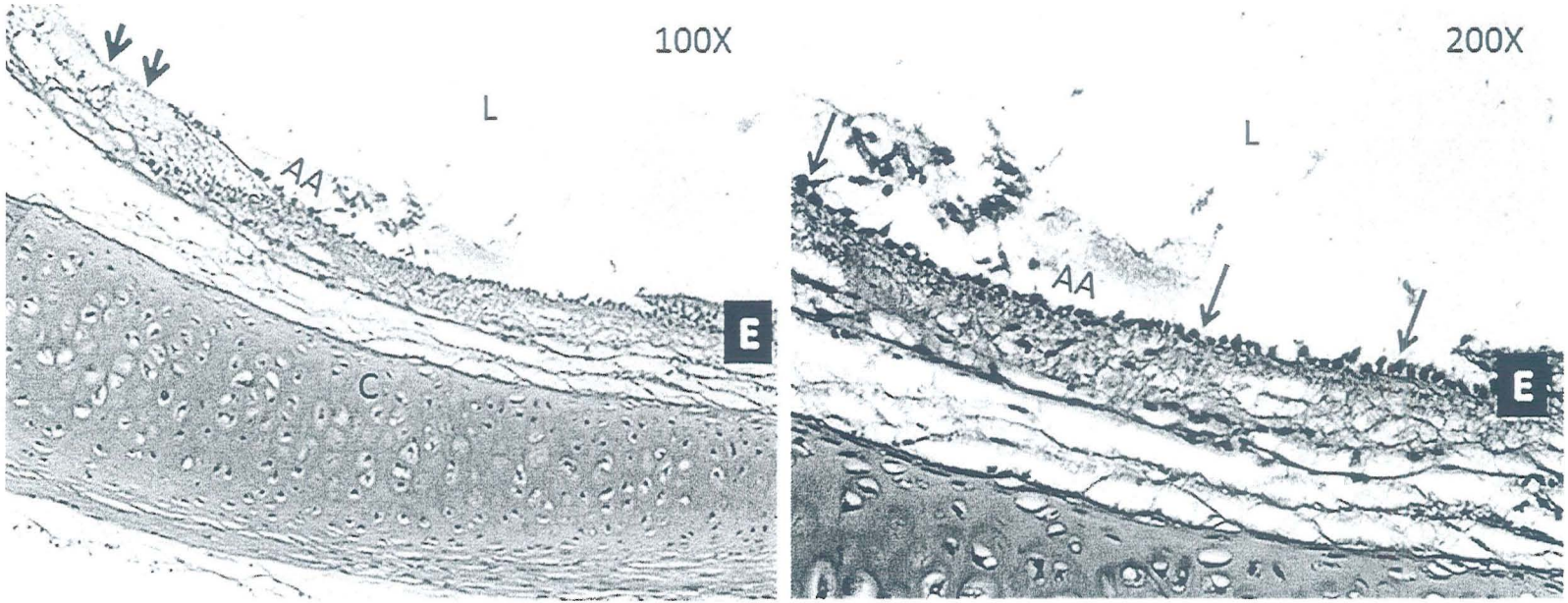


Figure 7. Twelve hours post injury. Non ciliated cells (thin arrow) covering the attenuated area under mining basement membrane. They are continually advanced from epithelial layer (E) and discontinued on the certain distance. Arrows show single layer of cells on the brushed area.

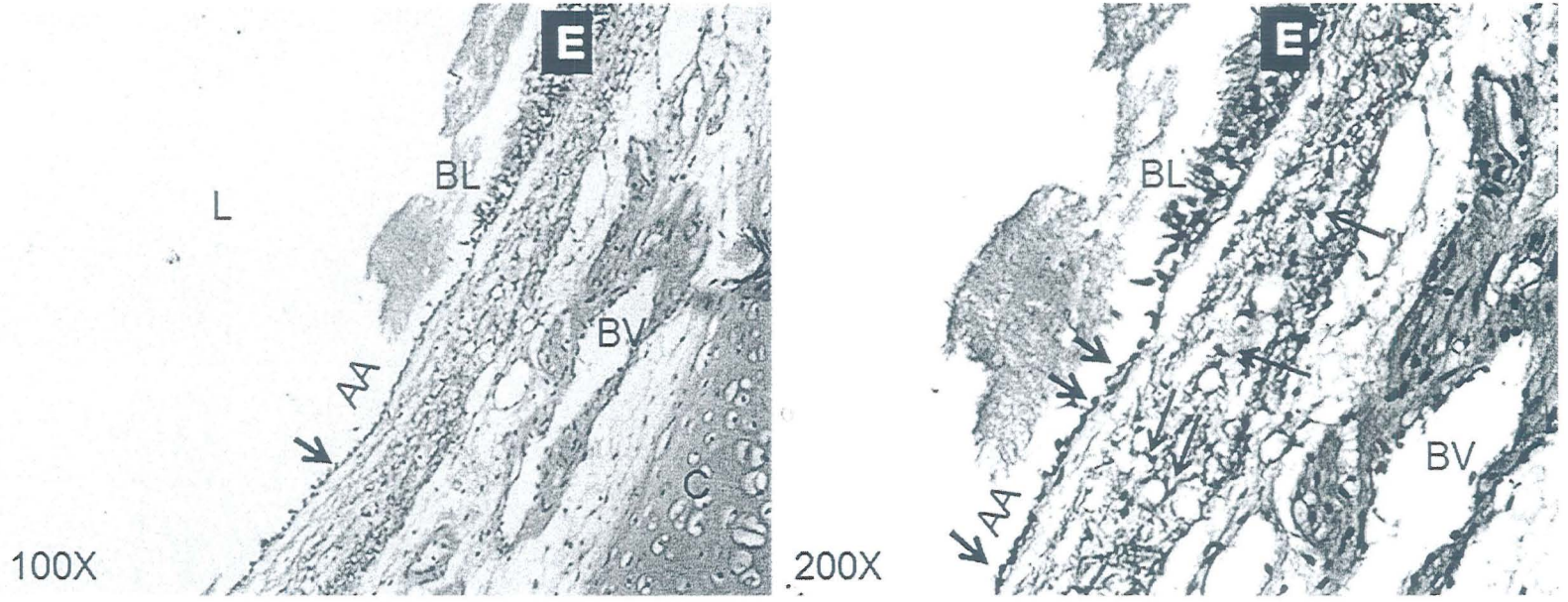


Figure 8. Twenty four hours post injury. Non ciliated cells (thick arrow) covering the brushed area. They are continually advanced from bordering the lesion (BL) toward the brushed area. Lymphocyte accumulating the submucosal region (thin arrow).

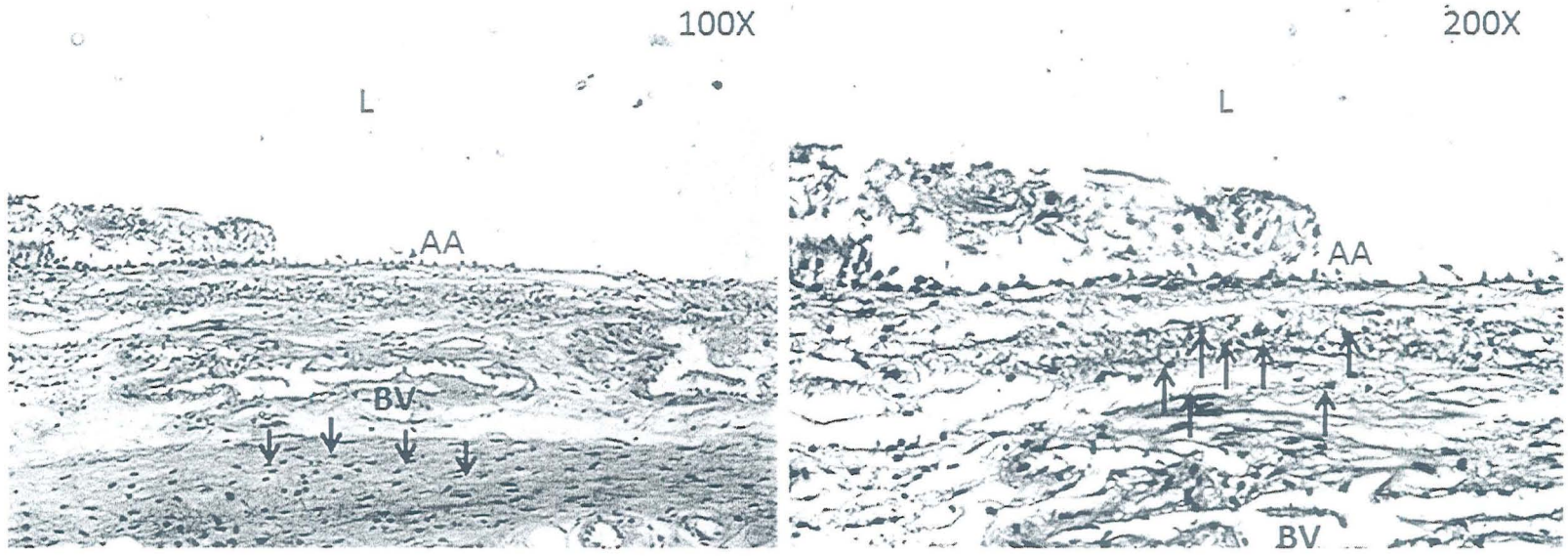


Figure 9. Forty eight hours post injury. Non ciliated cells (thick arrow) covering the damaged area. They are continually advanced from bordering the lesion (BL) toward the attenuated area. Lymphocytes are accumulating the submucosal region (thick arrow).

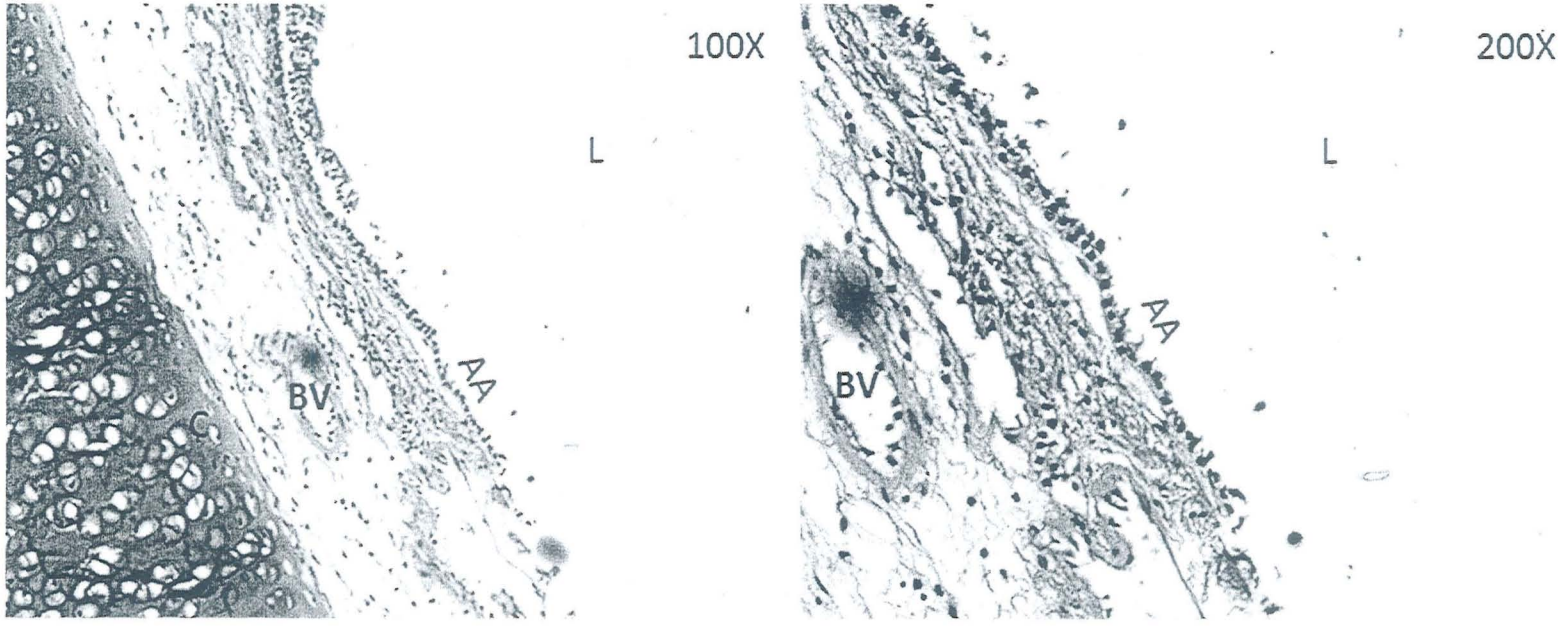


Figure 10. Seventy two hours post injury. Non ciliated cells (thick arrow) covering the brushed area. Large population of lymphocyte populated the submucosa area.

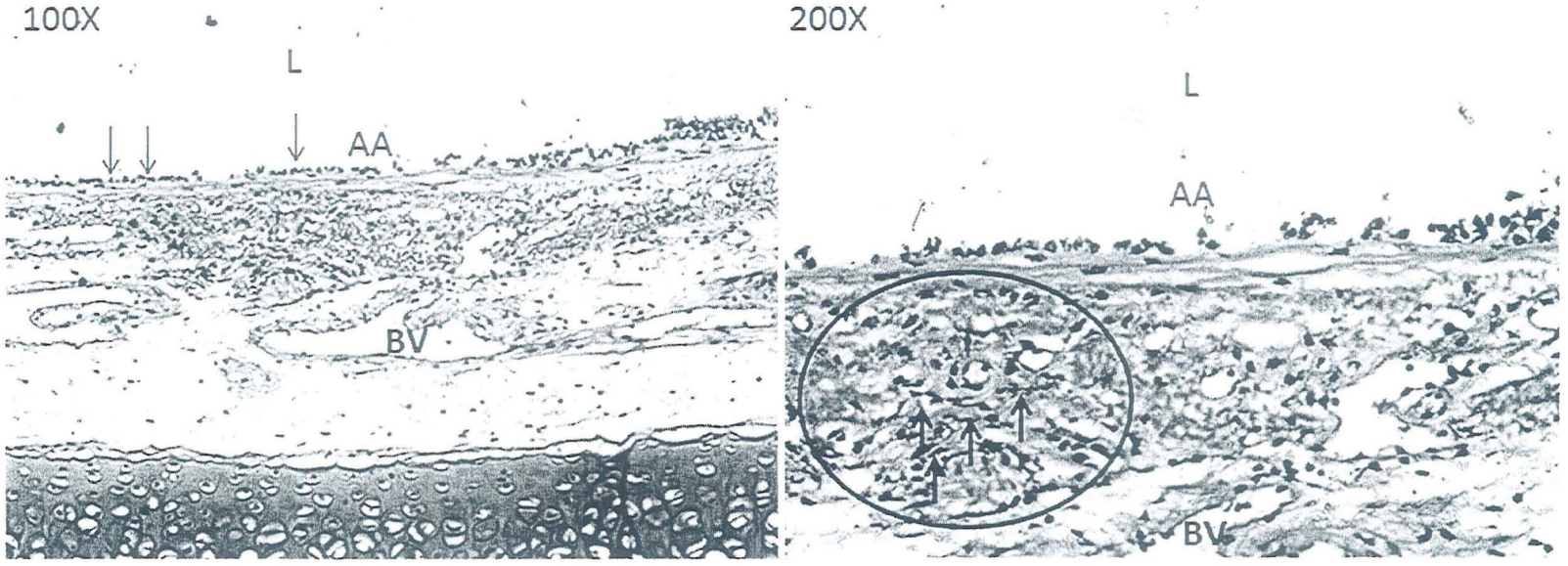


Figure 11. Ninety six hours post injury. The brushed is covered with non-ciliated cells (thin arrow). Large population of lymphocyte on the submucosa layer (thick arrow in the circle).

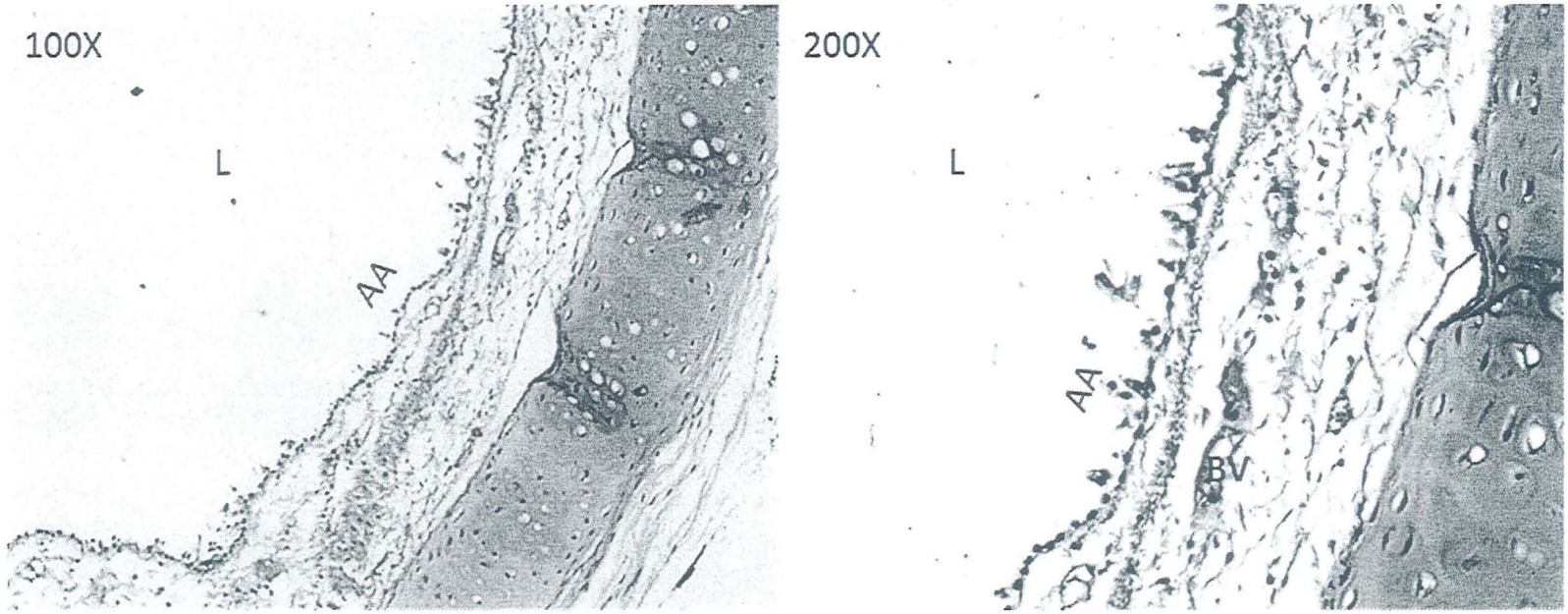


Figure 12. Seven days post injury. Damaged area covered with non-ciliated cells. Lymphocyte scattered around submucosal area.

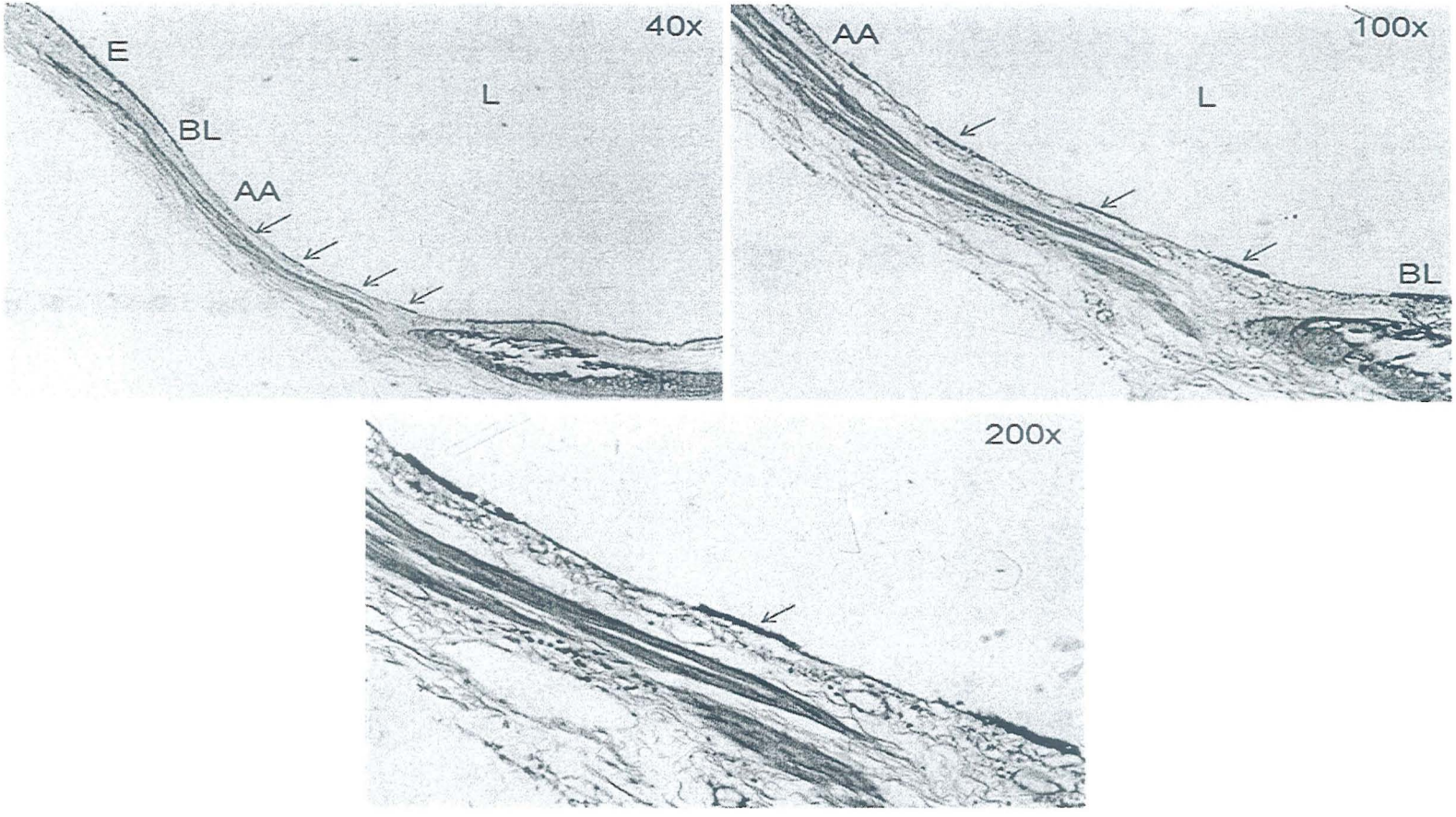


Figure 13. Fourteen days post injury. The bordering the lesion (BL) consists of a single layer of flattened cells. As it advanced from BL to the brushed area (AA), this layer reduces in thickness and discontinued before reach AA. A few of flattened cell groups organize scattered around the AA. None inflammatory cells seen in the submucosa area.

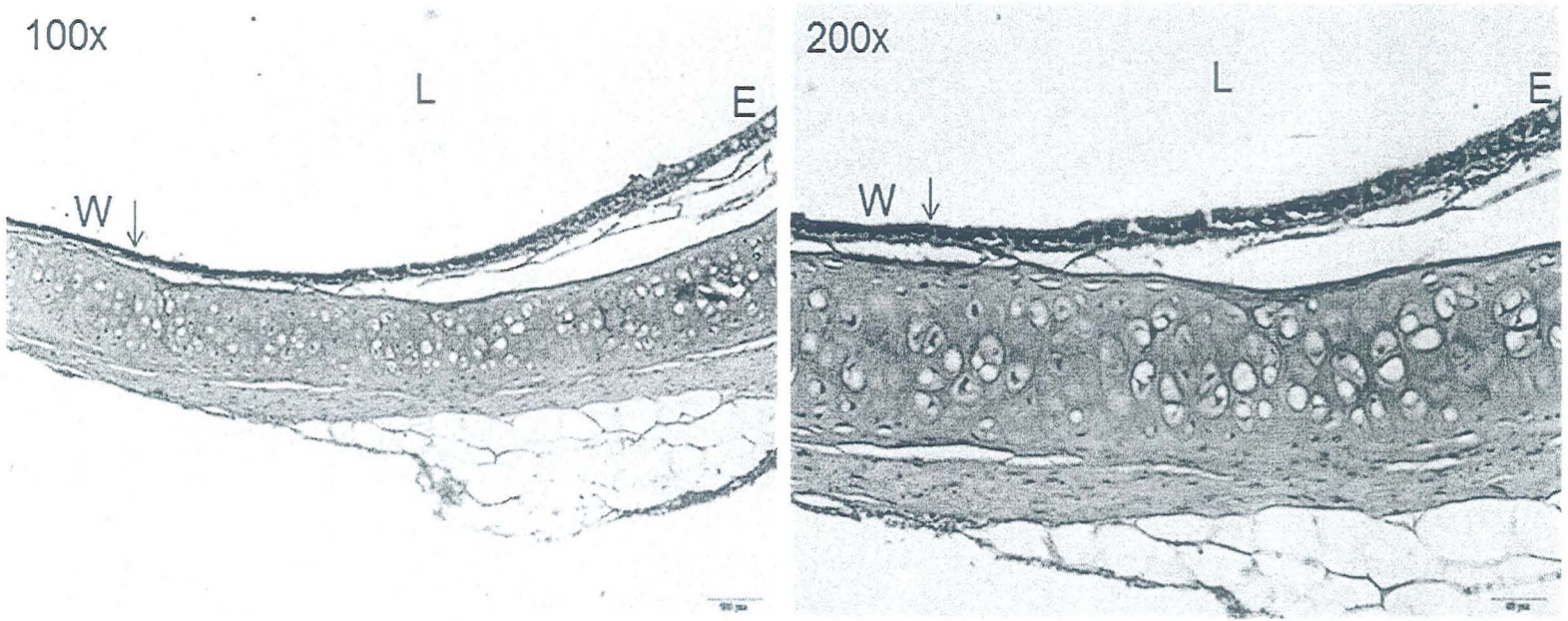


Figure 14. Twenty one days post injury. Epithelial layer (E) reduces in thickness and continuously attach with flattened cells (arrow) when advancing into wounded area. The wounded area (W) is fully covered with these flattened cells.

Discussion

Normal tracheal airway tissue consist epithelium, basement membrane, submucosa region and cartilage l. The epithelium layer has function as protection barrier from the irritant and foreign substance. They have defence mechanism by producing mucous, act as biological barrier, and motility clearance (Knight and Holgate, 2003; Puchelle et al., 2006). Any disturbances of this layer might compromise the physiological function of the trachea. Brush technique design to inflict injury by remove epithelial layer and leave intact layer adjacent to injured area. Using this technique enable to study the behaviour of the cell that located both on bordered lesion and denuded area. Consequent to this injury, epithelial cell will enters common repair process with the early stage cell at the bordered lesion migrate and spread to denuded area as a form squamous metaplasia. This process subsequently followed by proliferation, redifferentiation, ciliogenesis, and complete epithelial restitution (Crosby and Waters, 2010; Puchelle et al., 2006).

In additional to this, the inflicted injury may give different explanation on molecular level that interact and driven cell behaviour. Interactions between factors release by cell and extracellular matrix (ECM) component play major role in process of cellular repair mechanism. Interleukins, matrix metalloproteinases (MMP), integrins, and epidermal growth factor (EGF) family are crucial groups of molecular factors in regulate repairing pathway (Crosby and Waters, 2010) especially in reconstituting the basement membrane following injury.

Our current study showed that the lengths of the injured sites were reduced over the time points post injury and thus could be good indicator that the regeneration was activated as to restore the normal tracheal epithelium structure. However, due to short examination period for cellular regeneration to occur in this study (as time points end at 48 hours) fully regenerated epithelium is not possible to be measured in our current report. Although the newly invented technique was completely blinded in terms without prior knowledge on the location and position of the target site for the brushing, this study has shown that the brushing given to the animal a consistent as previously published (Yahaya B et al., 2011).

Previous method had certain limitation in term of practicality and time consuming due to mandatory of tracheotomy (Hilding, 1965; Kajstura et al., 2011; Keenan et al., 1982; Wilhelm, 1953). Exposing the trachea using tracheotomy allow researcher execute the technique. Additionally, this technique requires personal skill in handling surgical tool to make incision and sealed incised area and the animal facing a greater risk of infection. We had performed a new brushing technique with the introduction of intubation of endotracheal tube and excluding the tracheotomy which reducing the time required. Escaping tracheotomy makes this method more practical, conveniently performed and reduces the risk to get infection. Our technique also unarguably suitable to be applied in the simple setting animal laboratory without using sophisticated equipment. It required low cost tools to perform the preclinical studies for researchers that have no access to advanced and sophisticated tools to study the effect of induced-brushing on tracheal epithelium structure.

In conclusion, we had successfully developed a more practical, time-efficient, and less risk of infection of brushing techniques use to impose required injury of tracheal airway in order to study the changes occur at cellular level during epithelium repair following physical injury. Therefore, we propose this new technique to be used as an alternative approach as to study *in vivo* cellular mechanisms in airway regeneration following injury.

1. References

Boers, J. E., J. L. den Brok, et al. (1996). "Number and proliferation of neuroendocrine cells in normal human airway epithelium." Am J Respir Crit Care Med **154**: 758-763.

Dupuit, F., D. Gaillard, et al. (2000). "Differentiated and functional human airway epithelium regeneration in tracheal xenografts." Am J Physiol Lung Cell Mol Physiol **278**: L165-L176.

Hilding, A. C. (1965). "Regeneration of respiratory epithelium after minimal surface trauma." The Annals of Otology, Rhinology, and Laryngology **74**(4): 903-914.

Hulbert, W. C., S. F. Man, et al. (1989). "The response phase-the first six hours after acute airway injury by S02 inhalation: an in vivo and in vitro study." Scanning Microsc **3**(1): 369-378.

Keenan, K. P., J. W. Combs, et al. (1982). "Regeneration of hamster tracheal epithelium after mechanical injury. I. Focal lesions: quantitative morphologic study of cell proliferation." Virchows Archiv. B, Cell pathology including molecular pathology **41**(3): 193-214.

Kim, J. S., V. S. McKinnis, et al. (1997). "Proliferation and repair of guinea pig tracheal epithelium after neuropeptide depletion and injury in vivo." Am J Physiol **273**(6 Pt 1): L1235-1241.

McDowell, E. M., P. J. Becci, et al. (1979). "The respiratory epithelium. VII. Epidermoid metaplasia of hamster tracheal epithelium during regeration following mechanical injury." J Natl Cancer Inst **62**: 995-1008.

Nakagishi, Y., Y. Morimoto, et al. (2005). "Rabbit model of airway stenosis induced by scraping of the tracheal mucosa." Laryngoscope **115**(6): 1087-1092.

Shimizu, T., P. Nettesheim, et al. (1992). "Expression of cell type specific markers during rat tracheal epithelial regeneration." Am J Respir Cell Mol Biol **7**: 30-41.

Smiley-Jewell, S. M. and C. G. Plopper (2003). "Proliferation during early phases of bronchiolar repair in neonatal rabbits following lung injury by 4-ipomeanol." Toxicol Appl Pharmacol **192**(1): 69-77.

Wilhelm, D. L. (1953). "Regeneration of tracheal epithelium." The Journal of Pathology and Bacteriology **65**(2): 543-550.

Yahaya, B., A. Baker, et al. (2011). "Analysis of airway epithelial regeneration and repair following endobronchial brush biopsy in sheep." Experimental Lung Research **37**(9): 519-535.

691830: Your manuscript has been accepted

Thomas Liehr [bmri@hindawi.com]

Sent: 28 February 2013 5:04 PM
To: Badrul Hisham Yahaya
Cc: i8lith@mti.uni-jena.de; fatin_asyikhin@live.com; myself_blisster@yahoo.com; nurulainatikahk@gmail.com; zaeriars@gmail.com

Dear Dr. Yahaya,

The review of the Review Article 691830 titled "The rabbit as a model for studying lung disease and stem cell therapy," by Nurfatin Asyikhin Kamaruzaman, Egi Kardia, Nurulain 'Atikah Kamaldin, Ahmad Zaeri Latahir and Badrul Hisham Yahaya submitted to BioMed Research International, has been completed, and I am pleased to inform you that your manuscript has now been accepted for publication in the journal.

The publication process of your manuscript will be initiated upon the receipt of the electronic files. Please login to the Manuscript Tracking System at the link below using your username and password, and upload the electronic files of your final accepted version within the next 2-3 days.

http://mts.hindawi.com/author/691830/upload.files/

The electronic files should include the following:

1- Source file (Word or TeX/LaTeX).
2- Final PDF file of the accepted manuscript.
3- Editable Figure files (each figure in a separate eps/postscript/word file) if any, taking into consideration that tiff, jpg, jpeg, bmp formats are not editable.

Thank you again for submitting your manuscript to BioMed Research International.

Best regards,

Thomas Liehr
i8lith@mti.uni-jena.de

UNIVERSITI SAINS MALAYSIA

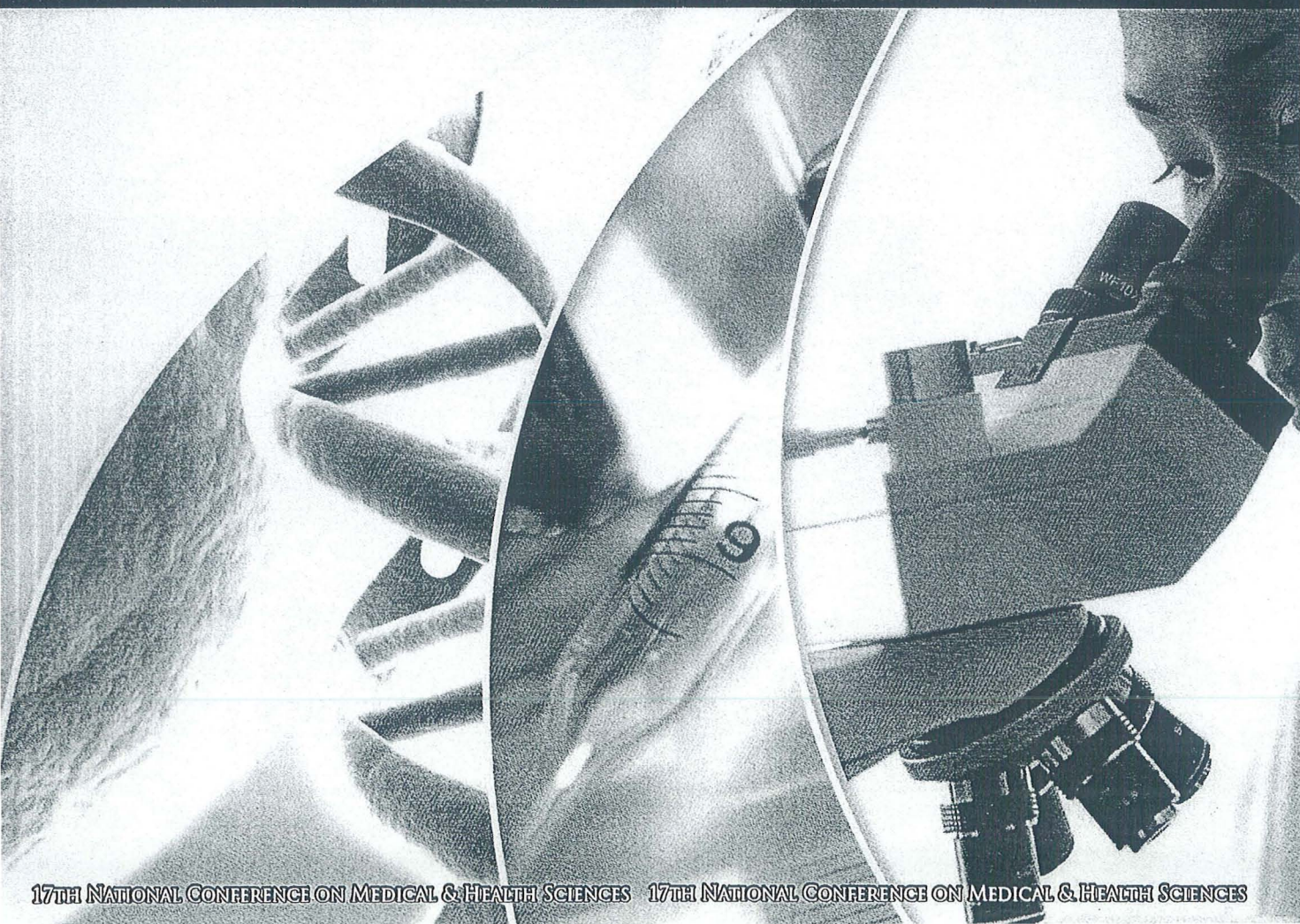
17th NATIONAL CONFERENCE ON MEDICAL AND HEALTH SCIENCES

Health Campus, Universiti Sains Malaysia, Kelantan

27th - 28th MAY 2012

Theme

Innovative Transformation: Elevating the Health of the Bottom Billion



PB11

TGF beta expression on diabetic wounds treated by *Momordica charantia* (MC) extract

Norhazilah Muhamad[1], Normaliza Omar[2], Teoh Seong Lin[2], Azian Abd.Latiff[2], Farida Hussan[2]

[1]Faculty of Medicine and Health Sciences, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia. [2]Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia.

Introduction: Wound healing property of *Momordica charantia* (MC) has been reported on diabetic animals in previous studies. Exogenous TGF beta has also been reported to induce wound healing.

Objective: To evaluate the TGF beta expression on the wound in diabetic rats treated with topical MC extract.

Methodology: Fifty-six male *Sprague-Dawley* rats were divided into 2 main groups: a non-diabetic group (*n*=6) and a streptozotocin-induced diabetic group (*n*=50). The diabetic groups were further subdivided into: a non-treated group (*n*=10), a treated group with MC powder (n=10), treated groups with or without MC ointment (*n*=10 each) and a povidine ointment treated group (n=10). The wound was inflicted with punch-biopsy needle on the dorsal aspect of thoracolumbar region. The wounds were treated for 10 days and the animals were sacrificed on day 11. The collected wound tissues were processed for immunohistochemical analysis with Dako REAL™ EnVision™ Detection System, Peroxidase/DAB, Rabbit/Mouse kit. The primary antibody, rabbit polyclonal to TGF beta (5 µg/ml) was used. DAB (3,3'-diaminobenzidin) served as chromogen (brown-red positive signal). Staining of normal dermal components and the duodenum tissue were served as positive controls and for standardization of the staining respectively. The TGF beta expression was measured qualitatively.

Results: The normal wounds showed higher expression of TGF beta compared to the diabetic wounds. Diabetic wounds treated with MC ointment showed higher intensity in expression of TGF beta compared to the other diabetic groups.

Conclusion: MC extract induces diabetic wound healing through expression of TGF beta expression.

PB12

Blinded brushing technique: a novel method to inflict injury on rabbit tracheal airway epithelium structure

Ahmad ZL[1], Yahaya B[2]

[1]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia. [2]Cluster for Regenerative Medicine, Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.

Introduction and objective: We reported a novel brushing technique to inflicting injury on the tracheal airway, termed blinded brushing technique using rabbit as a model for tracheal epithelium injury and repair.

Methodology: Rabbits were categorised as either treated with blinded-tracheal brushing or untreated as to serve as control for normal tracheal epithelium structure. We subsequently euthanized treated rabbits in different time points post-infliction in order to examine the effect of the brushing to the tracheal epithelium structure.

Results: Our results demonstrated that this technique was successfully removed the intact epithelium layer and its basement membrane without prior knowledge of the location of injury. The length of the induced-injuries were measured between the edges of the remaining epithelium bordering the lesion and the results found that the length of the injured areas were gradually decreased over the time points as compared to 30 min following injury. The decreases of the length of the injuries indicate that regeneration process was activated as to restore the normal epithelial layer.

Conclusion: As a conclusion, we had successfully developeda more practical and time-efficient brushing techniques with less infection risks and thus could be very useful technique in order to study cellular and molecular mechanisms during airway regeneration and repair.

Official Journal of TESMA
Regenerative Research
www.regres.tesma.org.my
E-ISSN 2232-0822

Tissue Engineering and Regenerative Medicine Society of Malaysia

Regenerative Research

www.regres.tesma.org.my
E-ISSN 2232-0822

4th Malaysian Tissue Engineering and Regenerative Medicine (MTERMS) Scientific Meeting

Theme: Gene, Cell and Tissue Therapy

June 3-4, 2012
Meritus Pelangi Beach Resort & Spa,
Pantai Cenang, Langkawi, Malaysia

Organised by:
TESMA & UPM

Volume 1
Supplement 1
June 2012

rabbit epidermal keratinocyte cultures, namely the Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), the CELLnTEC 07–Progenitor Cell Targeted media (Human/Mouse Keratinocytes-defined) and the CELLnTEC 57-Progenitor Cell Targeted media (Human/Mouse Keratinocytes-Low BPE). DMEM with 10% FBS retarded the growth of keratinocyte cultures. The CELLnTEC 07 medium resulted in 50% growth of keratinocytes whereas 90% of cell growth was observed with the CELLnTEC 57 medium. CELLnTEC 57 is the most suitable growth medium for the culture of rabbit epidermal keratinocytes in this study. This could be due to the low levels of BPE in the culture medium that favour the growth of rabbit epidermal keratinocytes. CELLnTEC 57-Progenitor Cell Targeted is a suitable growth medium for primary rabbit epidermal keratinocytes *in vitro*.

TP 08-50. Bioactive Fractions Isolated from a Plant to Improve Wound Healing in a Hyperglycemic Animal Model

Fakurazi S[1,2], Abu Bakar AH[1], Aimi SP[1], Dahiru S[1], Zalinah A[2], Faridah A

1 *Laboratory of Vaccine and Immunotherapeutics, Institute of Bioscience, Universiti Putra Malaysia*

2 *Laboratory of Vaccine and Immunotherapeutics Faculty of Medicine and Health Sciences, Universiti Putra Malaysia*

3 *Laboratory of Natural Product, Institute of Bioscience, Universiti Putra Malaysia*

The presence of wound and hyperglycemic conditions are complicated combinations. Following hyperglycemia, a small wound tends to become chronic which subsequently leads to permanent morbidity. Thus, the search for efficient wound healing products continues alongside the growing practice of traditional herbal medicine. The study was conducted to investigate the improvement of wound in hyperglycemic models following the application of bioactive fractions from *Moringacae* species. Crude extract was prepared in a powder form and various *in vitro* tests were conducted to evaluate the action of the fractions. Following isolation of the active fractions, active compounds were identified. The fraction was then tested on a wound-induced hyperglycemic animal model and the action of the fraction was evaluated. Following the closure of the wound, as compared to the control, the fraction was confirmed to have an active wound healing activity in a diabetic animal model.

TP 09-51. Analysis of Pathophysiological Changes in Regeneration and Repair of Rabbit Airway Tracheal Epithelium Response to Induced-Injury

Ahmad, Z.L[1*]., Tuan Sharif, S.E[2]., Zakaria, Z[3]., Yahaya, B[4].

1 *Human Genome Centre,* 2 *Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

3 *Cancer Research Centre, Institute for Medical Research (IMR), Kuala Lumpur*

4 *Cluster for Regenerative Medicine, Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas*

**Email:* azaerilatahir@yahoo.com

Understanding the mechanisms that operate during airway repair processes is fundamental to developing cell-based therapeutic approaches towards better treatment of lung-related diseases. The airway epithelial layer undergoes well-defined repair stages such as cell migration, proliferation, and redifferentiation in response to injury. In order to study the cellular mechanisms of response to injury and repair, we imposed an injury on the rabbit's trachea by performing a newly invented procedure called blinded-brushing technique that does not require specific surgery skills, time-efficient and less risk of infection as compared to induced epithelium injury. Rabbits were exposed to tracheal brushing and euthanized at different time points – 30 min, 1 hr, 6, 12, 24, 48, 72, 96 hrs and 7 days (n=3 for each time point including naive control animals). The length of the induced-injuries was measured between the edges of the remaining epithelium bordering the lesion. The results found that the length of the injured area was gradually decreased over the time points as compared to 30 min following injury. Decreases in the length of the injuries indicate that the regeneration process was activated to restore the normal epithelial layer. Various histopathological responses were observed from 30 min to day 7 post-brushing, from completely removal of the epithelium layer and its basement membrane until some of injured areas were covered by a series of single-cell layers at day 7. We have successfully developed a more practical and time-efficient brushing technique with less infection risks and thus could be a very useful technique in order to study cellular and molecular mechanisms during airway regeneration and repair that is comparable to studies that are carried out on larger animal models such as sheep and calves.

TP 10-58. Biocompatibility of Tobramycin-Incorporated Calcium Phosphate as Local Drug Delivery System

Che Nor Zarida CS[1], Fauziah O[2], Arifah AK[3], Azfar Rizal A[4], Nazri MY[1], Ahmad Hafiz Z[1], Rusnah M[5], Mohd Azam Khan GK[6], Hasni Idayu S[7], Rusliza Basir[2]

1 *Department of Orthopaedics, Traumatology and Rehabilitation, Kulliyyah of Medicine, IIUM, Jalan Hospital, 25150 Kuantan, Pahang.*
2 *Department of Human Anatomy, Faculty of Medicine and Health Sciences, UPM, 43400 Serdang, Selangor.*
3 *Department of Veterinary Preclinical Science, Faculty of Veterinary Medicine, UPM, 43400 Serdang, Selangor.*
4 *Department of Orthopaedics, Faculty of Medicine and Health Sciences, UPM, 43400 Serdang, Selangor.*
5 *Malaysian Nuclear Agency (Nuclear Malaysia), Bangi, 43000 Kajang, Selangor.*
6 *Faculty of Veterinary Medicine, UMK, Karung Berkunci 36, Pengkalan Chepa, 16100 Kota Bharu, Kelantan.*
7 *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, UPM, 43400 Serdang, Malaysia.*

The development of calcium phosphate as drug carrier is an important breakthrough in the field of bone repair biomaterials. Calcium phosphate minerals such as β-tricalcium phosphate (β-TCP) and dicalcium phosphate dehydrate (DCPD) have been shown to be suitable materials for local drug delivery in orthopaedic applications. The mineral component of calcium

Journal of Animal and Veterinary Advances 11 (20): 3772-3775, 2012
ISSN: 1680-5593

Blinded Brushing Technique as a Novel Method to Inflict Injury on Rabbit Tracheal Airway Epithelium Structure

Ahmad Z. Latahir and Badrul H. Yahaya
Cluster for Regenerative Medicine, Advanced Medical and Dental Institute (AMDI),
Universiti Sains Malaysia, Bandar Putra Bertam, 13200 Kepala Batas, Penang, Malaysia

Abstract: The normal response on the airway epithelial lining given injury comprises migration, proliferation and redifferentiation. Researchers reported here a novel brushing technique for inflicting injury on the tracheal airway, termed blinded brushing technique using rabbits as a model. Rabbits were categorised as either treated with blinded-tracheal brushing or untreated as to serve as control for normal tracheal epithelium structure. Researchers subsequently euthanized all rabbits in different time points (ranges from for 30 min and 1, 6, 12 and 24 h) post-infliction in order to examine the effect of the brushing to the tracheal epithelium structure. The results demonstrated that this technique was successfully removed the intact epithelium layer and its basement membrane without prior knowledge of location and position of the target site on the tracheal epithelium. The length of the induced-injuries were measured between the edges of the remaining epithelium bordering the lesion and the length of the injured areas were gradually decreased over the time points as compared to 30 min following injury. The decreases of the length of the injuries indicate that regeneration process was activated as to restore the normal epithelium layer. As conclusion, researchers had successfully developed a more practical and time-efficient brushing technique that could be very useful technique as to provoke cellular and molecular activations during airway regeneration and repair in respond to injury.

Key words: Blinded brushing technique, trachea, inflicted injury, cellular response, length of injury

INTRODUCTION

Brushing technique is a mechanical method utilised to inflict an injury to the tracheal epithelium layer. This technique is widely used in animal studies as to understand the cellular and molecular changes in response to injury which may potentially lead to identification of potential targeted cells that predominantly involve in airway regeneration and repair (Heguy *et al*., 2007; Kajstura *et al*., 2011). Current brushing techniques require surgical procedure in order to inflict injury on the tracheal airway (Nakagishi *et al*., 2005). Researchers used different brushing tools to expose and incise the trachea including steel probe, cotton swab and curettage (Hilding, 1965; Keenan *et al*., 1982; Wilhelm, 1953). Despite its wide utilization in research, the surgery-related techniques possess some disadvantages. Dedicated time and personnel with surgical skills are required since correct incision is mandatory. In addition, the surgical wound would potentially expose the animals with high risks of infection. Therefore, additional treatments are required to ensure the animals stay healthy and alive in order to study the effect of induced-injury on airway injury and repair. Thus, this study was aimed to develop a new tracheal-induced injury technique in order to increase the effectiveness of the procedure in inducing injury whilst reduce the risk of infection.

Researchers have developed a novel brushing technique which does not require surgical opening of the trachea. The novel technique is expected to overcome disadvantages incurred by the surgery-related techniques. It is no longer required to involve personnel with specific surgical skills and thus much shortcutting the procedure. Moreover, the absence of surgical wound would be expected significantly reduce the risk of infection to the animals. In this report, researchers demonstrated that the novel technique is capable of inflicting injury to the tracheal epithelium as per requirements in conducting such studies. The injury produced by this technique is comparable to earlier published techniques that involved either surgical or broncoscopic-based procedures as to study cellular and molecular changes during epithelium injury and repair.

Corresponding Author: Badrul Hisham Yahaya, Cluster for Regenerative Medicine, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, No. 84, Lot 45-2, Persiaran Seksyen 4/9 Bandar Putra Bertam, 13200 Kepala Batas, Penang, Malaysia

MATERIALS AND METHODS

New Zealand white rabbits (n = 21), weights ranging from 2-4 kg (2.7±0.6 kg) were used in this experiment. Rabbits were housed individually under standard condition before the experiment was conducted. Rabbits were grouped into normal (n = 3), sham treated (n = 3) and brushed (based on different time points): 30 min (n = 3), 1 (n = 3), 6 (n = 3), 12 (n = 3) and 24 (n = 3) h. Study protocol was approved by the Animal Ethics Committee of the Universiti Sains Malaysia (USM) (USM/Animal Ethics Approval/2010/63/258).

In brushed group, rabbits were anaesthetised with intramuscular injection of ketamine (35 mg kg^{-1}) (Troy ilium, Australia) and xylazine (Troy ilium, Australia) (3 mg kg^{-1}) (Fig. 1). Anaesthetised rabbits placed in supine position on surgical operating table. The palm pressed to ensure the rabbit was unconscious. The tongue of the rabbit was pulled aside to open the mouth wider. Endotracheal Tube (ET) (Grand, China), 2.5 mm in size and 8 cm in length was intubated into the trachea through mouth and confirmed by the presence of breathing sound. Interdental brush (Oral-B, US) with 15 cm in length was then inserted into ET. Twenty strokes of brushing was performed in 20 sec with 4 cm distance for each successful stroke (Fig. 1). Presence of bleeding on the interdental brush evidently shows the injury was occurred. After brushing was completed, the rabbit was put back into their cage before euthanized. Euthanasia was performed at 30 min, 1, 6, 12 and 24 h following brushing.

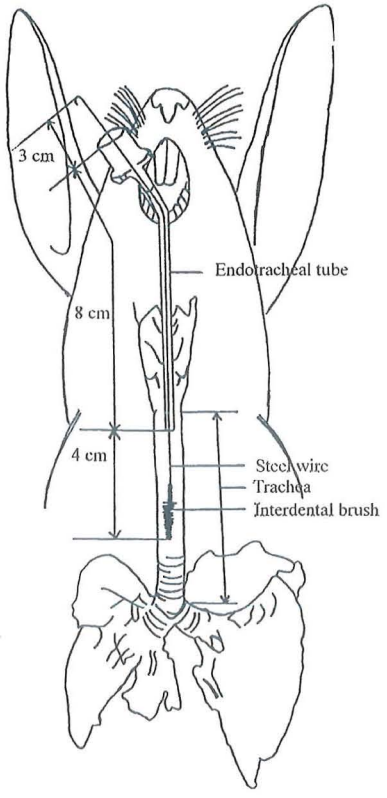


Fig. 1: Schematic diagram of the position and measurement of Endotracheal Tube (ET) and interdental brush during intubation and brushing procedures on rabbit

In sham-treated group, the tracheal perturbation was done using ET alone. The perturbation stop until the presence of breathing sound was noticed. In each successful perturbation, the ET was left for 20 sec. The ET was removed and rabbit was put back into the cage for 1 h before sacrificed. For untreated group, rabbits were euthanized without prior brushing.

Each rabbit was euthanized by giving an overdose of intravenous sodium pentobarbital (CEVA Sante Animale, France). Rabbit was placed in supine position with the hind and fore limbs spread laterally. The skin was cut and incised up to the anterior neck to expose the abdominal and thoracic cavities. Ribs were cut to expose the lungs and trachea. The trachea tissues were trimmed and fixed in 10% formalin solution for 24 h. The trachea was cut laterally into different section with approximately 0.5 cm thick. The tissues were processed for following procedures.

Each section was individually embedded in paraffin wax and cross sectioning into 5 μm thick using microtome (Lieca, Germany). The sections were subjected to standard haematoxylin and eosin (H&E) staining. The sections were viewed under light microscope (Olympus, US) and captured using image analyser software (Soft Imaging System Olympus, US). The present of injury was confirmed when the loss of the epithelial layer and/or its basement membrane were observed. Length of injury was measured between two edges of remaining epithelial layers bordering the lesion.

RESULTS AND DISCUSSION

The brushing was considered successful when pseudostratified epithelium and its basement membrane layer were absent as compared to the remaining epithelium bordering denuded area and intact epithelium on unbrushed tracheal tissues. The length of injury for every time point was measured and plotted in the graph (Fig. 2). The average length of the wounded areas was 244.6 μm (SD±172.9).

The histological manifestation of injury was shown by complete loss of the epithelium layer and/or its basement membrane. Loss of the epithelium layer was observed at all-time points post-injury and in some tissues the basement membrane still intact at the mucosa region. Severe damage was seen at 6 h post-injury as both the epithelium layer and its basement membrane were loss (Fig. 3).

Physical method such as brushing technique gives a force that causes disintegration of this layer. In the present study, the injury was found in both ET alone (sham-treated animal) and ET with interdental brush. However, in sham-treated group, the ET alone was found only causing a mild disruption on the epithelium layer with no evident of inflammatory responses following perturbation. This finding indicates that the ET alone was not sufficient to cause severe injury on tracheal epithelium especially on the submucosa layer. However, combination of ET and interdental brush performed on the animal was found to lead further destruction to area of submucosa region and blood vessels. Thus, bleeding was also considered as one of important indicators of the technique in order to confirm the injury was occurred.

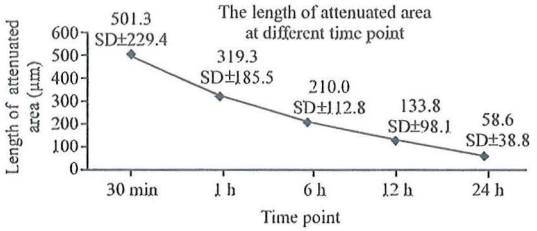


Fig. 2: The length of injury measured at 30 min, 1, 6, 12 and 24 h post brushing. The length of the injuries was measured between two remaining intact epithelium layers bordering the lesion. The length of injuries was decreased over the time points

Normal tracheal airway tissue consist epithelium, basement membrane, submucosa region and cartilage. The epithelium layer has function as protection barrier from the irritant and foreign substance. They have defence mechanism by producing mucous, act as biological barrier and motility clearance. Any disturbances of this layer might compromise the physiological function of the trachea. Brushing technique was designed to inflict injury by disrupting the intact epithelium layer thus provokes cellular responses not only cells reside bordering the lesion but also circulating cells, i.e., blood and/or bone marrow-derived cells. The involvement of cells bordering the lesion to migrate and spread to the area of injury will eventually promote cellular proliferation and redifferentiation as to reconstitute the loss of epithelium layer and to gain its normal function (Crosby and Waters, 2010).

In addition to this, the inflicted injury may give different explanation on molecular level that interact and

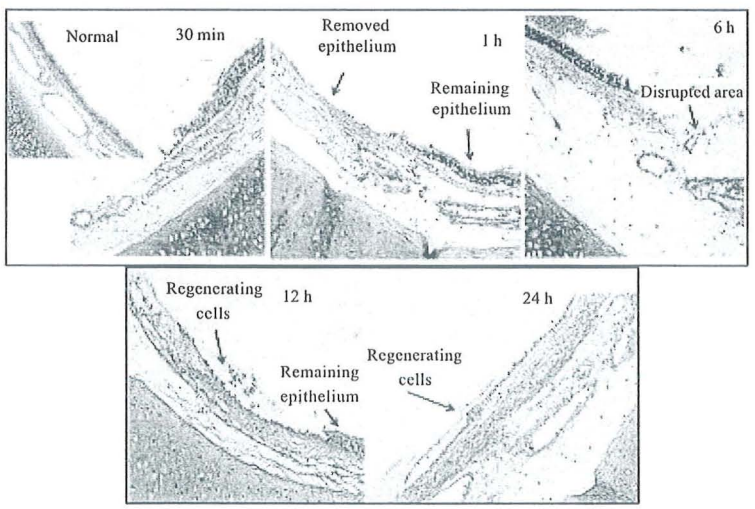


Fig. 3: The effect of blinded-brushing on the tracheal epithelium structure. The H&E staining was performed on the tissues collected at various time points ranges from 30 min, 1, 6, 12 and 24 h. The changes on tracheal epithelium structures were compared to normal tracheal epithelium (unbrushed rabbit). The brushing was successfully removed the normal structure of the tracheal epithelium, its basement membrane and the blood vessel was massively disrupted but the cartilage was remained intact. The inflammatory responses were clearly observed following at the later time points

driven cellular behaviour. Interactions between factors release by cell and Extracellular Matrix (ECM) component play major role in process of Cellular Repair Mechanism. Interleukins, Matrix Metalloproteinases (MMP), integrins and Epidermal Growth Factor (EGF) family are crucial endobronchial brush biopsy in sheep (Yahaya *et al.*, 2011).

Earlier methods had certain limitation in term of practicality and time consuming due to mandatory of tracheotomy (Hilding, 1965; Kajstura *et al.*, 2011; Keenan *et al.*, 1982; Wilhelm, 1953). Recently, another study was conducted using similar method to the proposed technique to produce chronic injury by repeated brushing on the trachea (Raub *et al.*, 2010). The injury was totally removed tracheal airway epithelium on the targeted areas. Contrary to this, repetition was not imposed in the method in which only single brushing was performed to produce an acute injury on the targeted site. This technique allows us to measure the length of injury between both edges of remaining epithelium layers. In addition to this it could also allow us to study on the role of remaining epithelial cells residing on the epithelium of bordering the lesion to dedifferentiate and migrate towards covering the denuded area in which these processes are important as to initiate cellular responses in airway epithelium regeneration and repair.

The technique also unarguably suitable to be applied in simple animal laboratory settings without using sophisticated equipment. It requires low cost tools to perform the preclinical studies for researchers that have no access to advanced tools in order to study the cellular and molecular mechanisms of airway epithelial cells in response to induced-brushing on tracheal epithelium structure.

CONCLUSION

Researchers had successfully developed practical and time-efficient with less risk of infection of brushing technique to induce tracheal epithelium injury in order to study airway epithelium regeneration and repair following physical injury. Therefore, researchers propose this new technique to be used as an alternative approach using rabbit as a model to study the effect of induced-injury on tracheal airway epithelium regeneration and repair.

ACKNOWLEDGEMENTS

Researchers would like to thank to staff of Animal Research and Service Centre (ARASC), USM and Central Research Laboratory (CRL), School of Medical Sciences, USM in helping us carried the experiment. This study was supported by USM Short Term Grant (304/PPSP/61311018) and USM Incentive Grant schemes.

REFERENCES

Crosby, L.M. and C.M. Waters, 2010. Epithelial repair mechanisms in the lung. Am. J. Physiol. Lung Cell Mol. Physiol., 298: L715-L731.

Heguy, A., B.G. Harvey, P.L. Leopold, I. Dolgalev, T. Raman and R.G. Crystal, 2007. Responses of the human airway epithelium transcriptome to *in vivo* injury. Physiol. Genomics, 29: 139-148.

Hilding, A.C., 1965. Regeneration of respiratory epithelium after minimal surface trauma. Ann. Otol. Rhinol. Laryngol., 74: 903-914.

Kajstura, J., M. Rota, S.R. Hall, T. Hosoda and D. D'Amario *et al.*, 2011. Evidence for human lung stem cells. N. Engl. J. Med., 364: 1795-1806.

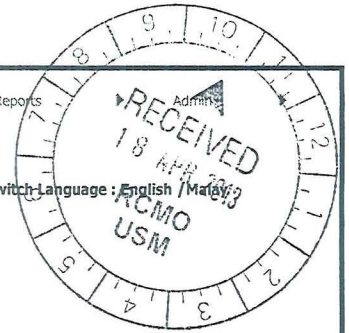
Keenan, K.P., J.W. Combs and E.M. McDowell, 1982. Regeneration of hamster tracheal epithelium after mechanical injury. I. Focal lesions: Quantitative morphologic study of cell proliferation. Virchows Arch. B Cell Pathol. Incl. Mol. Pathol., 41: 193-214.

Nakagishi, Y., Y. Morimoto, M. Fujita, Y. Ozeki, T. Maehara and M. Kikuchi, 2005. Rabbit model of airway stenosis induced by scraping of the tracheal mucosa. Laryngoscope, 115: 1087-1092.

Raub, C.B., S. Mahon, N. Narula, B.J. Tromberg, M. Brenner and S.C. George, 2010. Linking optics and mechanics in an *in vivo* model of airway fibrosis and epithelial injury. J. Biomed. Opt., Vol. 15, No. 1. 10.1117/1.3322296.

Wilhelm, D.L., 1953. Regeneration of tracheal epithelium. J. Pathol. Bacteriol., 65: 543-550.

Yahaya, B., A. Baker, P. Tennant, S.H. Smith, D.J. Shaw, G. McLachlan and D.D. Collie, 2011. Analysis of airway epithelial regeneration and repair following endobronchial brush biopsy in sheep. Exp. Lung Res., 37: 519-535.



Purchase Requisition ▸ Purchase Order ▸ Suppliers ▸ Maintenance ▸ Financials ▸ Coda Info ▸ Reports ▸ Admin ▸

UserCode: AZIAN / USMPGLIVE / CIPPT **Program Code: Votebook9100** **Current Program : Votebook (Header)**

Current Date : 18/04/2013 12:34:03 PM **Version: 15.03, Last Updated at 03/12/2012** **DB: 13.02, 9/27/2010 VB: 13.01, 3/14/2011** **Switch Language : English / Malay**

Wildcard : eg. Like 100%, Like 10%1, Like %1

Element 1: % Element 2: % Element 4: CIPPT

Element 5: 61311018 Year: 2013

Detail	Excel	Budget Rule	Budget Control	Account Description	Budget Account Code	Roll over	Budget	Cash Received	Advanced	Commit	Actual	Available	Percentage
Detail	Excel	1332	T	Projek Jangka Pendek Kelantan	304.221.0.CIPPT.61311018	1,200.00	0.00	0.00	0.00	0.00	0.00	1,200.00	0.00%
Detail	Excel	1332	T	Projek Jangka Pendek Kelantan	304.223.0.CIPPT.61311018	455.66	0.00	0.00	0.00	0.00	0.00	455.66	0.00%
Detail	Excel	1332	T	Projek Jangka Pendek Kelantan	304.227.0.CIPPT.61311018	3,626.92	0.00	0.00	0.00	0.00	0.00	3,626.92	0.00%
Detail	Excel	1332	T	Projek Jangka Pendek Kelantan	304.229.0.CIPPT.61311018	-5,144.20	0.00	0.00	0.00	0.00	0.00	-5,144.20	0.00%
		1332	T	SubTotal		138.38	0.00	0.00	0.00	0.00	0.00	138.38	0.00%
Detail	Excel	1335	T	Projek Jangka Pendek Kelantan	304.552.0.CIPPT.61311018	-60.96	0.00	0.00	0.00	0.00	0.00	-60.96	0.00%
		1335	T	SubTotal		-60.96	0.00	0.00	0.00	0.00	0.00	-60.96	0.00%
		9999		GrandTotal		77.42	0.00	0.00	0.00	0.00	0.00	77.42	0.00%