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

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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.

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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.

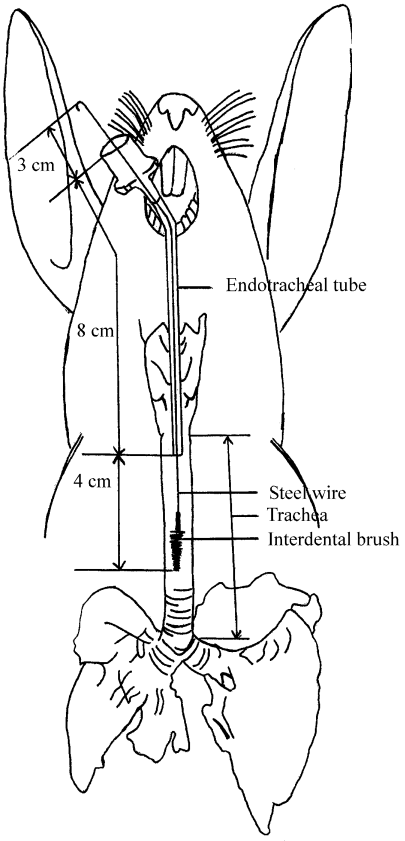

Fig. 1: Schematic diagram of the position and measurement of Endotracheal Tube (ET) and interdental brush during intubation and brushing procedures on rabbit

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.

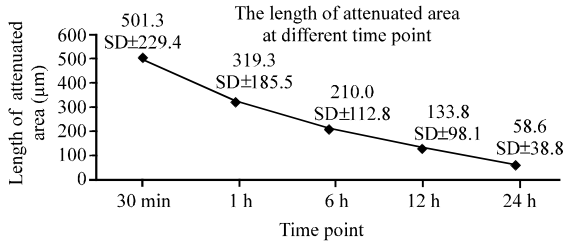

Fig. 2: The length of injury measured at 30 min, 1, 6, 12 and 24 h post brushing. The length of the injuries was measured between two remaining intact epithelium layers bordering the lesion. The length of injuries was decreased over the time points

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

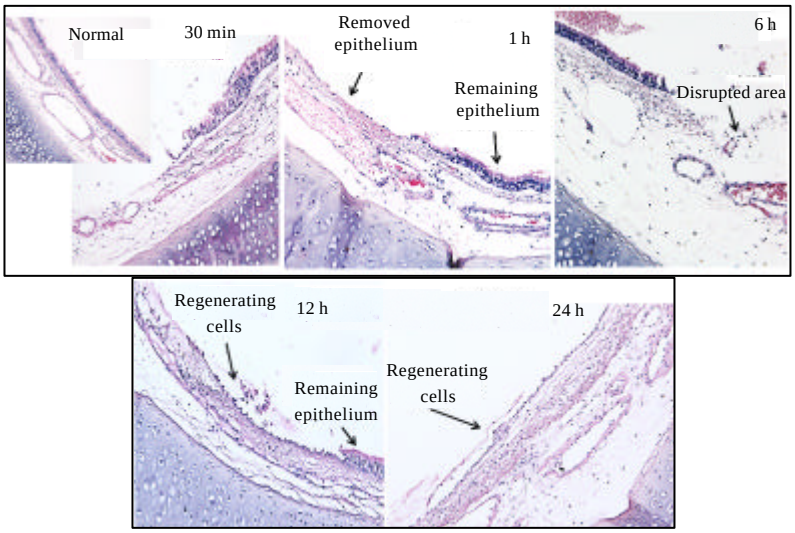

Fig. 3: The effect of blinded-brushing on the tracheal epithelium structure. The H&E staining was performed on the tissues collected at various time points ranges from 30 min, 1, 6, 12 and 24 h. The changes on tracheal epithelium structures were compared to normal tracheal epithelium (unbrushed rabbit). 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.

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