An electron microscopic study on the regeneration of the curetted tracheal mucosa in rats

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Summary

The mucosa of the respiratory tract, if desquamated by injury or infection, regenerates rapidly. The purpose of the present study is to demonstrate the morphological process of early stage respiratory mucosal regeneration in rats.

The tracheal mucosa of 54 adult rats of both sexes was removed by curetting through tracheostoma. Each time, from 1 hour to 12 hours after curetting, specimens were collected from the injured area of the trachea of 4 rats for TEM and SEM study. In addition, vascular network casts for SEM were made each time by infusion with artificial resin through the aortic arch of 2 rats.

1) As early as 2 hours after curetting, the surviving epithelial cells (mainly the basal cells) began to migrate from the wound margin. This migration was still continuing 12 hours after curetting, although no increase in mitosis was found yet in the surviving epithelial cells.

2) Also beginning one hour and 30 minutes after curetting, the injured blood vessels formed terminal “blind” branch vessels (mainly capillaries and veins). Four hours and 30 minutes after curetting, many bud-like processes were observed at the floor and margin of the curetted wound. Six hours after curetting, these processes grew into “blind” branch capillaries, and areas of these capillaries anastomosed with the neighboring capillaries or veins. Twelve hours after curetting, new vascular networks were formed in the areas of the new vessels (mainly capillaries and veins), but the arrangement of vessels was irregular. No increase of mitosis was found in the lamina propria and submucosal layer within 12 hours after curetting.

Key words: rat, tracheal mucosa, regeneration, electron microscope

Introduction

“The wound healing process” is one of the oldest study themes for surgeons. Yet, there remain many unknown aspects of the healing process. As otolaryngologists, we
frequently observe that the respiratory tract mucosa, if desquamated by injury or infection, regenerates again rapidly. There have been numerous of morphological (especially electron microscopic) studies dealing with the healing of injured respiratory tract mucosa; however, these studies are mainly concerned on the epithelium.

The purpose of this study is to observe the early stage of the morphological processes of mucosal regeneration in the injured respiratory tracts of rats. Both the epithelia and the submucosal vascular networks of rat tracheas were studied.

**Materials and Methods**

The animals used were 60 adult albino rats of both sexes, weighing 200 g each. 6 rats were used as controls. In 54 rats, the anterior and both lateral mucosal walls of the tracheas were removed by curetting. During the 12 hours following the treatment groups of 6 rats were sacrificed and each group was separated into a group of 4 and a pair. Each group of 4 rats was used for TEM study of the tracheal mucosa, as follows. The tracheas of each of the 4 rats were quickly removed, washed and fixed in ice-cold 2.5% cacodylate buffered glutaraldehyde solution. The tracheas were cut into appropriate size, postfixed in ice-cold 1% cacodylate buffered osmium tetroxide solution, and then were dehydrated in ethanol. The dehydrated tracheas of 2 of these 4 rats were embedded in EPON for TEM study of the epithelial layers. The ultrathin sections were stained with uranyl acetate and lead citrate and observed with TEM (model Hitachi HS9).

The dehydrated tracheas of the other 2 of the 4 rats were used for SEM study of the epithelial cell surfaces. Isoamyl acetate was used for transferring from ethanol to liquid carbon dioxide in the critical point drying apparatus (model Hitachi). The dried specimen surfaces were coated with gold and observed with TEM (model Akashi Minisem).

The pair of rats from each original groups of 6 were used for SEM study of the vascular networks of the tracheal mucosa. The vascular network casts were made each time by perfusion with artificial resin (Melcox; made by Dainihon Ink) through the aortic arch, as follows. On each rats, immediately after sacrifice, ligations were made in two places on the intravenous catheter (7 Fr.) introduced into the aortic arch through an incision in the left ventricle. The right ventricle was also incised. The whole body was perfused with heparinized Ringer’s solution and then perfused with 2.5% cacodylate buffered glutaraldehyde solution. After fixation a ligation was made on the descending aorta and the upper half of the body was perfused with 10 ml of artificial resin. Following the polymerization of the resin, the trachea was carefully removed and macerated at room temperature in 20% potassium hydroxide. Each cleaned and dried vascular network cast was coated with gold and observed with SEM (model Akashi Minisem II).

**Results**

1. *The regeneration of the epithelial layer*

As early as 2 hours after curetting, the surviving epithelial cells of the curetted
wound margin began to migrate centripetally onto the floor of the wound. The migrating epithelial cells consisted mainly of basal cells. These cells were flat in shape and arranged in layers of one, two, or more. Immature ciliated cells and immature secretory cells were already observed within the migrating epithelial cells 3 hours after curetting. The migrating epithelial cells were observed until 12 hours after curetting.

In due time course, the area of the migrating epithelial cells increased. The number of immature ciliated cells and of immature secretory cells within the migrating epithelial cells also increased. No increase in mitosis was observed in the migrating epithelial cells or in the surviving epithelial layer from 1 to 12 hours after curetting.

2. The reconstruction of the vascular network of the submucosal layer

The submucosal vessels of a normal trachea in the rats consisted of veins, capillaries and arteries. These vessels anastomosed with each other and formed regularly-arranged vascular networks with longitudinally-oriented meshes. The capillaries were arranged at the superficial layer, the veins were arranged at the inner layer, and the arteries ran through the venous plexuses, dividing into smaller arterioles, anastomosed with the capillaries and veins. Within 1 hour and 30 minutes after curetting, the healing process of the injured vascular network had already begun. One hour and 30 minutes after curetting, the injured blood vessels had stopped hemorrhaging and formed terminal “blind” branch vessels consisting mainly of capillaries and veins. Three hours and 30 minutes after curetting, many “bud-like-processes” were observed at the floor and margin of the curetted wound. These processes germinated from the intact capillaries and veins. With the time course, the “bud-like-processes” increased in number, and some of them grew into new terminal “blind” branch capillaries. On the other hand, punctured “bud-like-processes” were sometimes observed as well. Six hours after curetting some of the terminal “blind” branch capillaries anastomosed with neighboring capillaries and veins. Twelve hours after curetting the new terminal “blind” branch capillaries and the new terminal “blind” branch veins had extended. The anastomosis of the regenerating vessels increased in number, and locally the formation of the new vascular network was observed, though the arrangement of the vessels was irregular. No increase in mitosis was observed in the walls of the vessels or other connective tissue cells, at either the curetted area or in the non-curetted area, from 1 to 12 hours after curetting.

Discussion

In general, regeneration of epithelial tissues is characterized by cycles of three processes: cell migration, proliferation, and differentiation. Authors have reported that in the respiratory tract the regeneration of an injured pseudostratified ciliated epithelium begins first with migration; next proliferation occurs, and finally differentiation occurs. These processes progress regularly, and regeneration is completed by the repetition of these processes. In our recent observations during 12 hours following curetting, the regeneration of the epithelial cells was certainly due to the migration of the surviving
epithelial cells; and, these migrating epithelial cells consisted mainly of the basal cells. However, these migrating epithelial cells contained some immature ciliated cells and some immature secretory cells during the whole course of observation. Therefore, it can be assumed that among the basal cells of the migrating epithelial cells, there are basal cells which have decided the course of their differentiation before migration begins. And, in the early stage of the regeneration of the injured tracheal epithelium, both migration and differentiation are progressing simultaneously.

Although there are some reports on the vascular networks of both the respiratory tract mucosa and other organs in normal and abnormal conditions morphologically, there is no report on the reconstruction of the injured vascular network of the respiratory tract mucosa without other organs. The morphological study of the reconstruction of injured tracheal mucosal vascular networks at an early stage is probably the only recent observation. The formation of terminal "blind" branch vessels was the first reaction of the injured vascular network. These "blind" branch vessels suggest a complete stopping of hemorrhage. Next, many "bud-like-processes" appeared at the floor and margin of the wound. These processes germinated from the intact capillaries and veins. These "bud-like-processes" were probably the earliest stage of the regenerating capillaries. Punctured "bud-like-processes" were sometimes observed as well. It can be speculated that the puncture of "bud-like-processes" is an artifact, probably caused by the high pressure of infusion and by the weakness of the walls of the "bud-like-processes". In general, walls of injured vessels are repaired with neighboring endothelial cells, free mesenchymal cells (fibroblasts, histiocytes and others), and intravascular elements. In our recent observation, the "bud-like-processes" and the new "blind" branch vessels were surrounded by endothelial cells and basal lamina; but, sometimes there were gaps along the endothelial cells of the walls of the "bud-like-processes". It can be concluded that at an early stage (from 1 to 12 hours after curetting), the walls of the "bud-like-processes" and of the new "blind" branch vessels consist only of the migrating endothelial cells from the surviving vessel walls.

References


Legends

Photo 1 One hour after curetting: on curetted wound margin some epithelial cells (probably basal cells: BC) survive. Basal lamina (BL) is observed clearly, it remains or renews. Lamina propria (LP) is intact. TEM, ×2300

Photo 2 Two hours after curetting: migrating epithelial cells (MEC) are observed on wound margin. MEC are flat and arrange in one or two layers. Basal lamina runs ahead of MEC (clearly marked by arrow). Edema is observed in lamina propria. TEM, ×2300

Photo 3 Three hours after curetting: ciliated cells and lysosomenrich-cells appear in migrating epithelial cells. MEC are still flat or cuboid. Many leucocytes (LC) as well as edema is observed in lamina propria. TEM, ×2300

Photo 4 Four hours after curetting: regenerating epithelial cells (migrating epithelial cells: MEC) migrate under crust (CR). Almost all of MEC consist of non-ciliated cells. Locally, ciliated cells (CC) are observed. SEM, ×1000

Photo 5 Four hours after curetting: high magnification of regenerating epithelial cells. Probably these cells are basal cells. SEM, ×10000

Photo 6 Vascular network cast of normal tracheal mucosa in rat. It consists of mainly capillaries and veins. All vessels anastomose the neighboring vessels. Arrangement of vessels is regular. SEM, ×200

Photo 7 One hour and 30 minutes after curetting: injured vessels of curetted wound recover by "blind" branch vessel formation (Arrows). They consist of capillaries and veins. SEM, ×200

Photo 8 Four hours and 30 minutes after curetting: many bud-like-processes appear in margin and floor of curetted wound. They germinate from capillaries and veins. SEM, ×200

Photo 9 Many bud-like processes appear in the margin and floor of a curetted wound. Some of them are punctured. ×2000

Photo 10 High magnification of bud-like-process of a single capillary. SEM, ×5000

Photo 11 Three hours after curetting: a red blood cell (RBC) protrudes through a gap in endothelial cells (EC) and basal lamina (BL) into the stroma (ST). TEM, ×12000

Photo 12 Four hours after curetting: a regenerating capillary (RCP) is surrounded by basal lamina, but along the outer wall of the RCP, the endothelial wall has a gap. TEM, ×12000

Photo 13 Four hours and 30 minutes after curetting: on the surviving capillary wall a leucocyte (LC) protrudes into the stroma, surrounded by intact endothelial cell and basal lamina. This is probably the “bud-like-process” formation. TEM, ×12000

Photo 14 Four hours and 30 minutes after curetting: a regenerating capillary (RCP) is observed in the surviving capillary wall. This regenerating capillary has intact endothelial cells and basal lamina. TEM, ×12000

Photo 15 Six hours after curetting: bud-like-processes increase in number and in size. Locally, bud-like-processes grow new "blind" branch capillaries and anastomose with neighboring capillaries or veins (Arrows). SEM, ×200

Photo 16 Twelve hours after curetting: new "blind" branch vessels (capillaries and veins) increase in number and in size (Arrows). SEM, ×200

Photo 17 Twelve hours after curetting: locally, new vessels form new vascular network: though arrangement of vessels is irregular. SEM, ×200

TEM: transmission electron microscope
SEM: scanning electron microscope

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