Ultrastructure of bacteria adhesion in cell membrane of young mouse lingual mucosa

Ii-Sei WATANABE[1]
Koichi Ogawa [2]
Marcelo Cavenaghi Pereira da SILVA[3]
Eduardo Shigueaki KADO[1]

1 - Department of Anatomy, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo - Brazil
2 - Department of Anatomy, School of Medicine, Fukuoka University, Fukuoka, Japan
3 - Department of Anatomy, Sao Leopoldo Mandic Research Center, Campinas - Brazil

Correspondência para:
II-SEI WATANABE
Departamento de Anatomia
Instituto de Ciências Biomédicas
Universidade de São Paulo
Av. Prof. Lineu Prestes, 2415 - Cidade Universitária
05508-900 – São Paulo – SP
watanabe@icb.usp.br

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Abstract

Lingual mucosa of young mouse was examined by transmission and high resolution scanning electron microscopic images (HRSEM). The specimens were fixed with modified Karnovsky solution and embedded in Spurr resin for transmission electron microscopy. Thin sections of 80 nm were cut and examined in the Jeol 1010 transmission electron microscope. For HRSEM method, the specimens were fixed in the same solution, postfixed in osmiun tetroxide, critical point dried and coated with palladium. The samples were examined under Hitachi S-900, SEM microscope. The results revealed groups of bacteria attached to the surface of keratinized epithelial cells. These streptococcus and coccus attached on the cell membrane were noted in the three-dimensional SEM images. At high magnification, the transmission electron microscopic images demonstrated the adhesion of bacteria to the cell membrane through numerous fimbria comprising the glycocalyx. The fine fibrillar structure rising from bacteria were clearly seen.

Key-words:
Tongue.
Mice.
HRSEM.
TEM.
Bacteria

Introduction

The tongue and palatine mucosa of several species including the human were studied employing scanning electron microscopic (SEM) and transmission electron microscopic (TEM) techniques. SEM techniques were utilized by Watanabe et al.[1] and Apleton and Tyldesley [2] in the rat; Iwasaki and Wanichanon[3] in the frog *Rana cancrivora*, Iwasaki and Sakata[4] in the bull frog, Iwasaki and Miyata[5] in the guinea pig, Iwasaki, Assami and Wanichanon[6], Iwasaki, Yoshizawa and Kawahara[7], 11 in the hawksbill turtle and rat snake, Yoshioka and Muto[8] in the rat and Watanabe[9] in the mouse.

There are few studies concerning the adhesion of bacteria on the epithelial cell membrane as described by Brady, Gray and Lara-Garcia[10]; Howlett and Squier[11]; McCourtie and Douglas[12]; Watanabe, Jin and Nagata[13]; Motoyama et al. [14] and Vitkov et al.[15].

This paper shows the presence of groups of bacteria and their adhesion to epithelial cell membrane of young mouse tongue mucosa employing the high resolution scanning electron microscopy (HRSEM) and transmission electron microscopy (TEM).

Materials and Methods

Eight young mice were anesthetized with sodium pentobarbital (30mg/Kg) and perfused with modified Karnovsky fixative solution containing 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cacodilate buffer (pH 7.3). The specimens were collected and immersed in the same fixative for 12 h at 4ºC. For high resolution scanning electron microscopy (HRSEM), the tissues were postfixed in 2% osmium tetroxide solution for 2 hs at 4º C, rinsed

with distilled water and then, in 2% tannic acid aqueous solution for 1 h at room temperature according to the technique described by Murakami[16]. The samples were rinsed in distilled water for 5 h, and postfixed with 2% osmium tetroxide solution for 2 hs at 4º C. They were dehydrated in series of ethanol and tert-buthyl alcohol, freeze-dried in Eiko ID-2 apparatus, mounted and coated with palladium in a BIO-RAD (SEM Coating System - Microscience Division, Japan). The samples were examined in a high resolution scanning electron microscope Hitachi, S-900 at 10 kV.

For transmission electron microscopy, the samples were fixed in the same solution, postfixed in 1% osmium tetroxide solution for 12 h at 4ºC, dehydrated in series of ethanol and propylene oxide and embedded in Spurr resin, according to Watanabe and Yamada[17]. Thick sections were made using glass knives and stainResults

Samples of young mice lingual mucosa examined at high resolution scanning electron microscope (HRSEM) showed in three-dimensional aspects the adherence of groups of bacteria in the epithelial cell membrane (Figura 1). Bacteria were present in depressions of microvilli and groups of coccus and staphilococcus were located in several regions of filiform and fungiform papillae. Bacteria were distributed randomly (Figura 1) or in rows (Figura 2). At high magnification, HRSEM images showed an elongated form of bacteria with a sulcus in the cell membrane (Figura 3).

The TEM showed several layers of keratinized epithelial cells and the surface of mouse tongue epithelium where numerous bacteria were attached (Figura 4 and 5). These bacteria were usually disposed in two rows (Figura 6). In face of that, the fine filamentous structure containing glycocalyx permitted the adhesion of bacteria between each other and to the surface of epithelial cells (Figura. 5, 6 and 7). At high magnification, the meshwork of fine fibrillar material around the surface ed with toluidine blue to choose the areas of interest under light microscopy. Thin sections were made ultramicrotome Ultra-Cut Reichert with diamond knife. The ultrathin

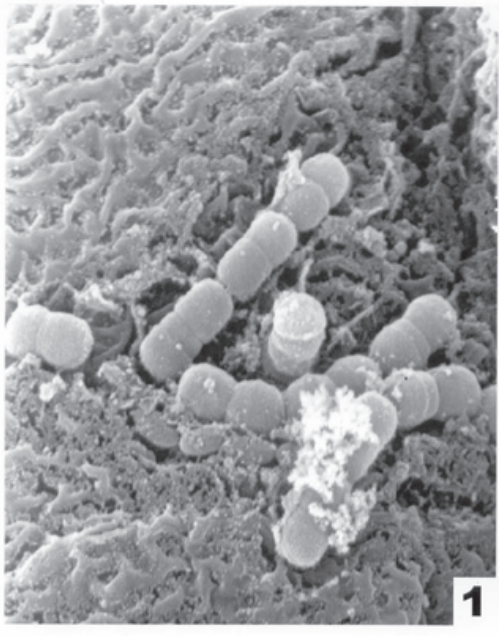

Figure 1 - HRSEM image of young mouse tongue. The surface of papilla shows the bacteria groups in three-dimensional images. X 22,000

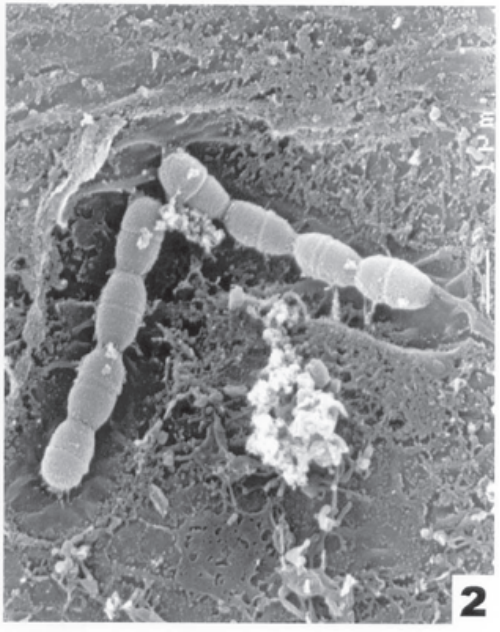

Figura 2 - HRSEM image of bacteria disposed in two rows. X 20,000

sections of 90 nm were made and counterstained with uranyl acetate and lead citrate and examined in transmission electron microscopy Jeol, 1010 at 80 kV. of bacteria and between the bacteria and cell membrane was clearly seen in figures 6, 7, and 8.

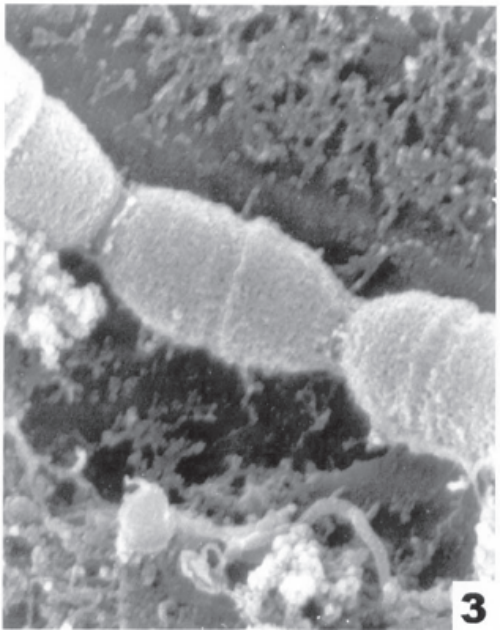

Figure3 - High magnification image of bacteria with sulcus and small granules. X 70,000

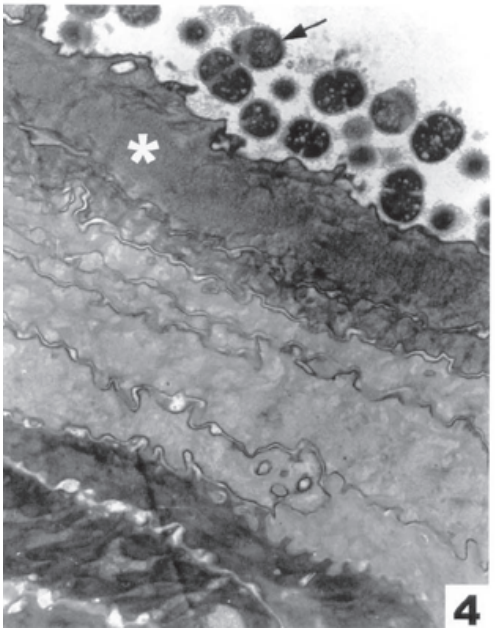

Figure 4 - TEM image of keratinized epithelial layer (*) revealing groups of bacteria (arrow). X 7,200

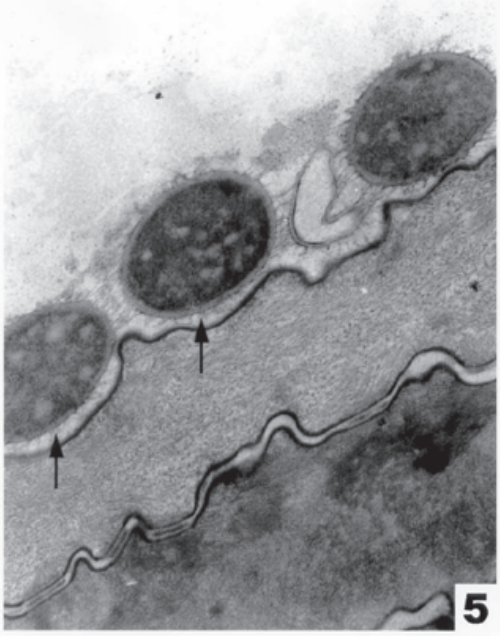

Figure 5 - At high magnification, the adhesion area between bacteria and cell membrane are clearly shown (arrows). X 36,000

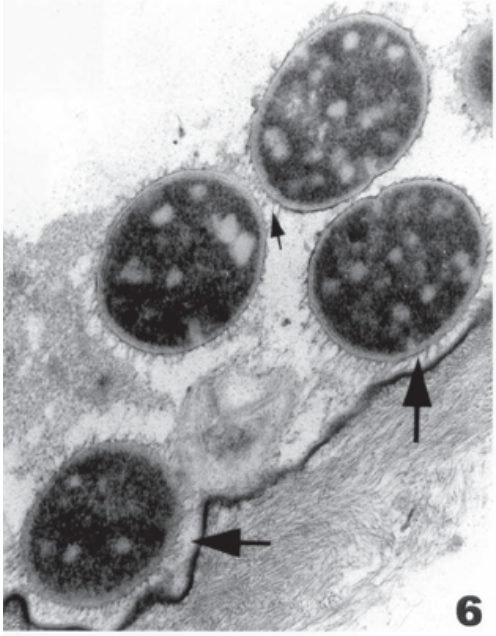

Figure 6 - High magnification shows the filamentous material between bacteria (small arrow) and between the bacteria and cell membrane (large arrows). X 40,800

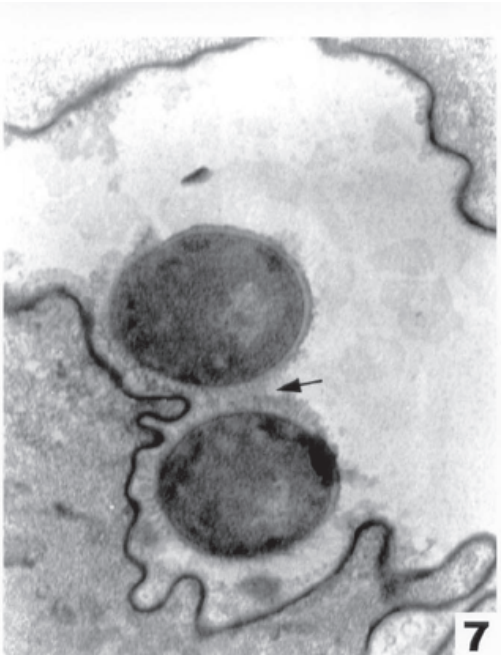

Figure 7 - At high magnification shows the adhesion of two coccus (arrow) in the depression of keratinized epithelial cells. X 45,000

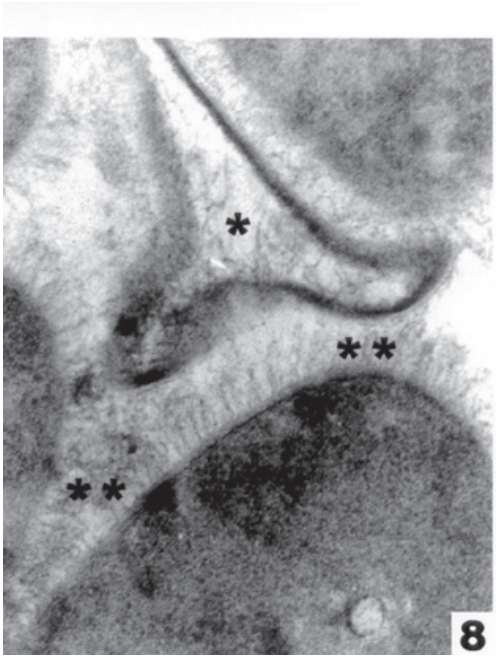

Figure 8 - Observe the filamentous material between the cytoplasmic extension (*) and membrane of bacteria and between the bacteria (**). X 90,000

Discussion

Our results demonstrated clearly the surface of epithelial cells and their microvilli with HRSEM images and microplicae using TEM. These characteristics are similar to those reported by Iwasaki and Sakata[4]; Yoshioka and Muto[8] and Watanabe[9] in the tongue mucosa of mammals.

The presence of groups of microorganisms attached to cell membrane in the surface of mouse tongue epithelial cell as demonstrated by HRSEM images were similar to the ones reported by Brady, Gray and Lara-Garcia[10] in the filiform papillae of rat tongue, by Vitkov et al. [18] in human oral mucosa and Watanabe et al.[1] in the anterior third of young rat tongue. Our results also demonstrated that the microorganisms are attached on the epithelial cell membrane of the papillae randomly through numerous fibrils structure. Barnett [18] suggested that the adhesion of the streptoccocae to cells may occur in face of the fibrillar structure complex formed by glycocalyx.

In our results were not observed bacteria penetrating into the epithelial layer as mentioned by Brady, Gray and Lara-Garcia[10]. However, the presence of microorganisms occurred in the depression of epithelial cells. The attachment of bacteria to epithelial cell surface occurred through an interaction between fibrillar substance and the epithelial cell membranes as demons-trated in our TEM images and the ones made by Tokunaga et al. [19] and Vitkov et al. [15]. Also, the ultrastructural findings concerning the, adhesion of *Candida albicans* were noticed by Howlett and Squier[11] and Tokunaga, Kusa-michi and Koike[20] and according to Critchley and Douglas[21]; Mccourtie and Douglas[22,12], there are numerous factors suggesting that cell membrane proteins are the most important molecule in the *Candida* adhesion. Our transmission electron microscopy and HRSEM data confirmed that between bacteria surface and the epithelial cell membrane there is a complex network of filamentous material.

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Ultraestrutura da adesão de bactérias na membrana celular da mucosa lingual de camundongos jovens

Resumo

A mucosa lingual de camundongos jovens foi examinada através de imagens de microscopia eletrônica de transmissão e de varredura de alta resolução. Os espécimes foram fixados em solução modificada de Karnovsky e emblocadas em resina Spurr para a microscopia eletrônica de transmissão. Cortes finos de 80 nm foram feitos e examinados em um microscópio eletrônico de transmissão Jeol 1010. Para a microscopia eletrônica de varredura de alta resolução os espécimes foram imersos na mesma solução, pós fixados em tetróxido de ósmio, secos e cobertos com paládio. As amostras foram examinadas em um microscópio eletrônico de varredura Hitachi S-900. Os resultados revelaram grupos de bactéria aderidos à superfície queratinizada das células epiteliais. Estes estreptococos e cocos aderidos à membrana celular foram notados em imagens tridimensionais. Em aumentos maiores, as imagens de microscopia eletrônica de transmissão demonstraram a adesão de bactéria à membrana celular através de numerosas fimbrias compondo o glicocalice. A estrutura fibrilar emergindo da bactéria foi claramente observada.

Palavras-chave:
Língua.
Camundongo.
Bactéria.
MET.
MEV.

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