

## Three-Dimensional Characteristics of Bacteriae in the Filiform and Fungiform Papillae of Rat: a TEM and HRSEM Study

Características Tridimensionales de Bacterias en las Papilas Filiformes y Fungiformes de rata: un Estudio en MET y ARMEB

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WATANABE, I.; OGAWA, K.; GUIMARÃES, P. J. & IYOMASA, M. M. Three-Dimensional characteristics of bacteriae in the filiform and fungiform papillae of rat: a TEM and HRSEM study. *Int. J. Odontostomat.*, 2(1):95-99, 2008.

**ABSTRACT:** Tongue mucosae of one day postnatal rat was examined by transmission electron microscopic and HRSEM methods. The specimens were fixed using Karnovsky solution and embedded in Spurr resin for transmission electron microscopy. For HRSEM methods, the samples were fixed in 2% osmium tetroxide, dehydrated in alcohol, critical point dried and coated with gold-palladium. The results demonstrated that the surface of tongue present the filiform and fungiform papillae covered by numerous keratinized epithelial cells. The bacteriae are attached to the surfaces of microplacae at random, demonstrating in their three-dimensional HRSEM images. At high magnification, the transmission electron microscopic images revealed the adhesion of bacteriae to the cell membrane by glycocalyx. The fibrillar structures surrounding the bacteriae are clearly seen.

**KEY WORDS:** epithelial cell, tongue, rat, coccus, bacillus, bacteriae, HRSEM, TEM.

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### INTRODUCTION

The bacteriae are located on the surface of oral mucosae but the adhesion may occur in different regions of epithelial surface. The epithelial cell characteristics have been studied in animals by Apleton & Tyldesley (1971), Iwasaki & Sakata (1985), Iwasaki & Wanichanon (1993), Iwasaki *et al.* (1996a, 1996b), Yoshioka & Muto (1976) using transmission electron microscopic methods.

On the other hand, Barnett (1973) reported the adhesion of the streptococcae cells on the epithelial cell surface mentioning that there are a complex structure formed by proteins. Brady *et al.* (1975) revealed the characteristic of attachments of the microorganisms in the outer surface of queratinized epithelial cells. Tokunaga *et al.* (1986, 1990) identified that the attachment of microorganisms to epithelial cell surface may occur through an interaction between fibrillar substance and the cell membranes. Although

Howlett & Squier (1980) and Tokunaga *et al.* (1986) reported the adhesion of *Candida albicans* and the epithelial cell surface. Also, in recent studies of the adhesion of bacteriae of epithelial cells are demonstrated by Watanabe *et al.* (1997; 1999, 2006) and Motoyama *et al.* (1999).

The present paper describe the presence of the coccus and bacillus on the cell membranes of filiform and fungiform papillae of one postnatal day of rat tongue employing the high resolution scanning electron microscopy (HRSEM) and transmission electron microscopy (TEM).

### MATERIAL AND METHOD

Ten rat tongues of one postnatal day were fixed by immersion using 2% osmium tetroxide buffered with

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sodium phosphate solution in a 1/15M (pH 7.4) at 4 °C during 2 h. Then, the tongues were rinsed in sodium phosphate buffer for over night, followed the postfixation in 2% osmium tetroxide aqueous solution and immersed in 2% tannic acid aqueous solution for 90 minutes at room temperature according to the method reported by Murakami (1974). The samples were washed again in distilled water for at least 3 hs, and postfixed with 2% osmium tetroxide solution for 2 hs at 4°C. The samples were dehydrated in increasing series of ethanol, and dried in critical point dryer ID-2 Eiko apparatus, and mounted on a metal lamina using carbon paste. They coated with gold-palladium in ion beam sputter Eiko, Japan, and the samples were examined in a high resolution scanning electron microscope Hitachi S-900 at 10kV.

For transmission electron microscopy, the tissues were fixed by immersion in modified Karnovsky solution for 3 h at room temperature, and then, postfixed in 1% buffered osmium tetroxide solution during 2 h at 4°C. The dehydration was made in an increasing series of ethanol and propylene oxide and embedded in Spurr resin, according to the technique reported by Watanabe & Yamada (1983). The thick sections of 0.5µm were made using ultramicrotome Ultra-Cut Reichert with glass knives and stained by toluidin blue solution. The sections were examined by light microscopy in order to localize the groupings of bacteriae. For thin sections were used ultramicrotome Ultra-Cut Reichert with diamond knife. The ultrathin sections of 90 nm thickness were mounted on 200 "mesh" with Formvar films and counterstained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in transmission electron microscopy Jeol, 1010 at 80 kV.

## RESULTS

The dorsal mucosa of one postnatal day of rat tongue were examined by high resolution scanning electron microscope (HRSEM) showed the filiform (Fig. 1) and fungiform papillae (Fig. 2). On the surface of epithelial cells of filiform papillae may be noted the keratinized epithelial cells with the adherence of groupings of bacteriae (Figs. 3 and 4). The fungiform papillae presenting polygonal epithelial cells showed a three-dimensional HRSEM images (Fig. 2).

The coccus and bacillus are attached at random on the epithelial cell membranes (Figs. 5 and 6). The groupings of bacteriae are variable in size essentially

on the filiform papillae surfaces as clearly showed in figures 5, 6, 7. At high magnification, the HRSEM images showed a round and elongated in shape of bacteriae and the surface of coccus presenting numerous small particles on the cell membranes (Fig.8). Between the cell surface may be noted the filamentous structure in three-dimensional images.

Transmission electron microscopy observations showed that the surface of epithelial cells of filiform and fungiform papillae of tongue may present the bacteriae. They are distributed at random and attached on the cell membranes (Fig. 9). The TEM images of bacteriae revealed a meshwork of fine fibrillar structure around of surface (Fig. 10).

## DISCUSSION

Our results demonstrated that one postnatal day of rat showed numerous filiform papillae presenting the groupings of bacteriae revealing in three-dimensional HRSEM images. The filiform papillae containing keratinized epithelial cells showed the membranes with micropliae which are similar to those demonstrated by Arvidson (1976); Iwasaki & Sakata (1985); Yoshioka & Muto and Watanabe. Although our results confirm that the surface of epithelial cell of one postnatal day of rat tongue revealed the groupings of with different numbers of microorganisms which are attached on the cell membranes as clearly demonstrated by HRSEM images and TEM images. The characteristics of presence the bacteriae on the queratinized epithelial cells of filiform papillae of rat adult tongue were identified by Brady *et al.*; and the adhesion of the streptococcus on the cells by Barnett, in the fungiform papilla of mouse by Watanabe *et al.* (1997); in the tongue of young rat by Motoyama *et al.* (1999); Watanabe *et al.* (1999) and in the young mice by Watanabe *et al.* (2006). In our ultrastructural observations revealed that the bacteriae presented the adhesion area on the epithelial cell membrane forming a interaction between fibrillar components and the surface of micropliae. It is important to emphazise that the proteins of the streptococcus may provide the adherence structure of the coccus and bacillus in the filiform and fungiform papillae. Similar findings were noted in the paper reported by Tokunaga *et al.* (1986,1990).

Although, the penetration of the coccus and bacillus depend on the localization and the epithelial

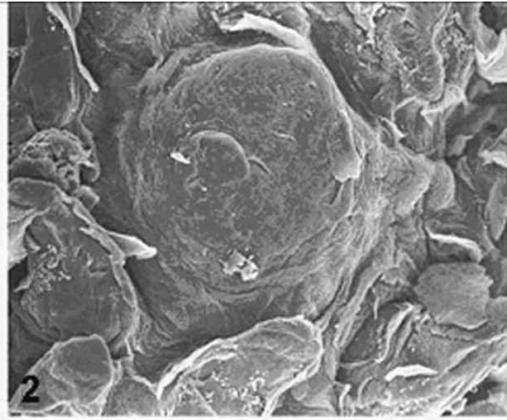
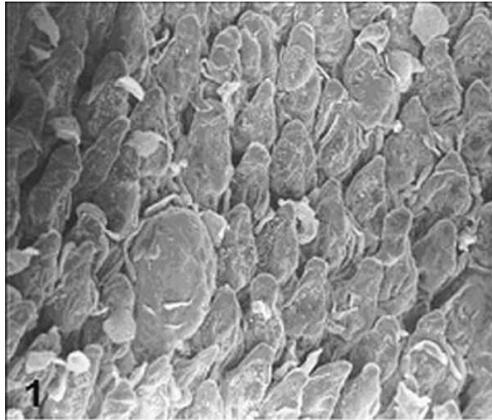


Fig.1 HRSEM image of one postnatal day rat tongue, revealing the filiform papillae in three-dimensional images. X 300.

Fig.2 HRSEM image of fungiform papilla showing the polygonal keratinized epithelial cells. X 710.

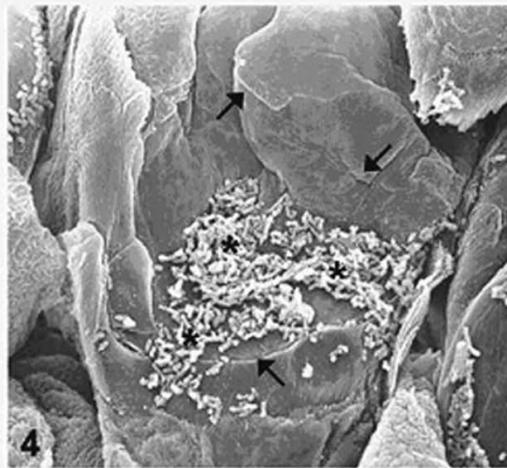
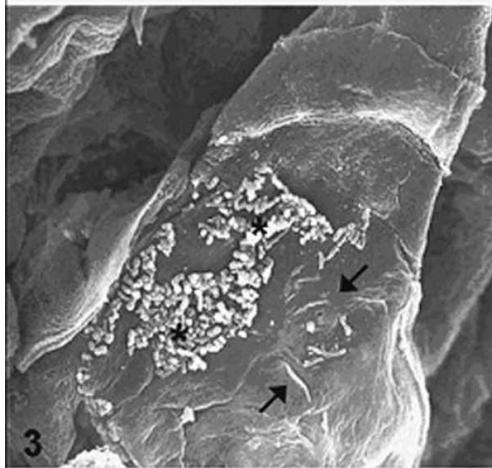


Fig.3 HRSEM image of filiform papilla showing numerous bacteriae (\*) attached to the keratinized epithelial cell surface (arrows). X 2.500.

Fig.4 HRSEM image of filiform papilla showing the groupings of bacteriae on the keratinized epithelial (arrows) at random (\*). X 2.000.

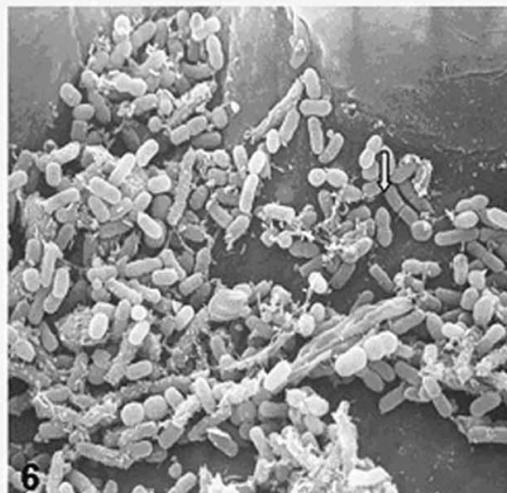
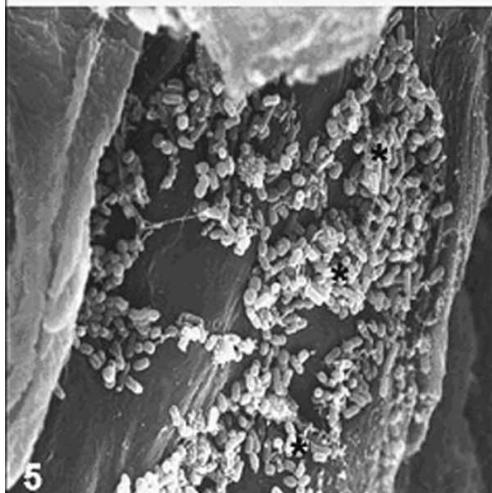


Fig.5 HRSEM image shows the surface of epithelium and groupings of bacteriae (\*). X 4.000.

Fig.6 HRSEM images of adhesion of numerous bacteriae, coccus and bacillus, are noted. X 7.000.

cell membrane features. In our results, the one postnatal day rat tongue revealed the microorganisms, essentially coccus and bacillus, were found on the surface of epithelial cells. Our findings using TEM methods confirmed that between bacteria surface and the microplicae of epithelial cells there are a complex network of filamentous and amorphous materials. We

believe that the method of high resolution scanning electron microscopy is very important to analyse the epithelial cells with the presence of microorganisms and their details of normal and pathological conditions. Other papers will be necessary using different methods to elucidate the aspects of the adhesion of the microorganisms in oral mucosa.

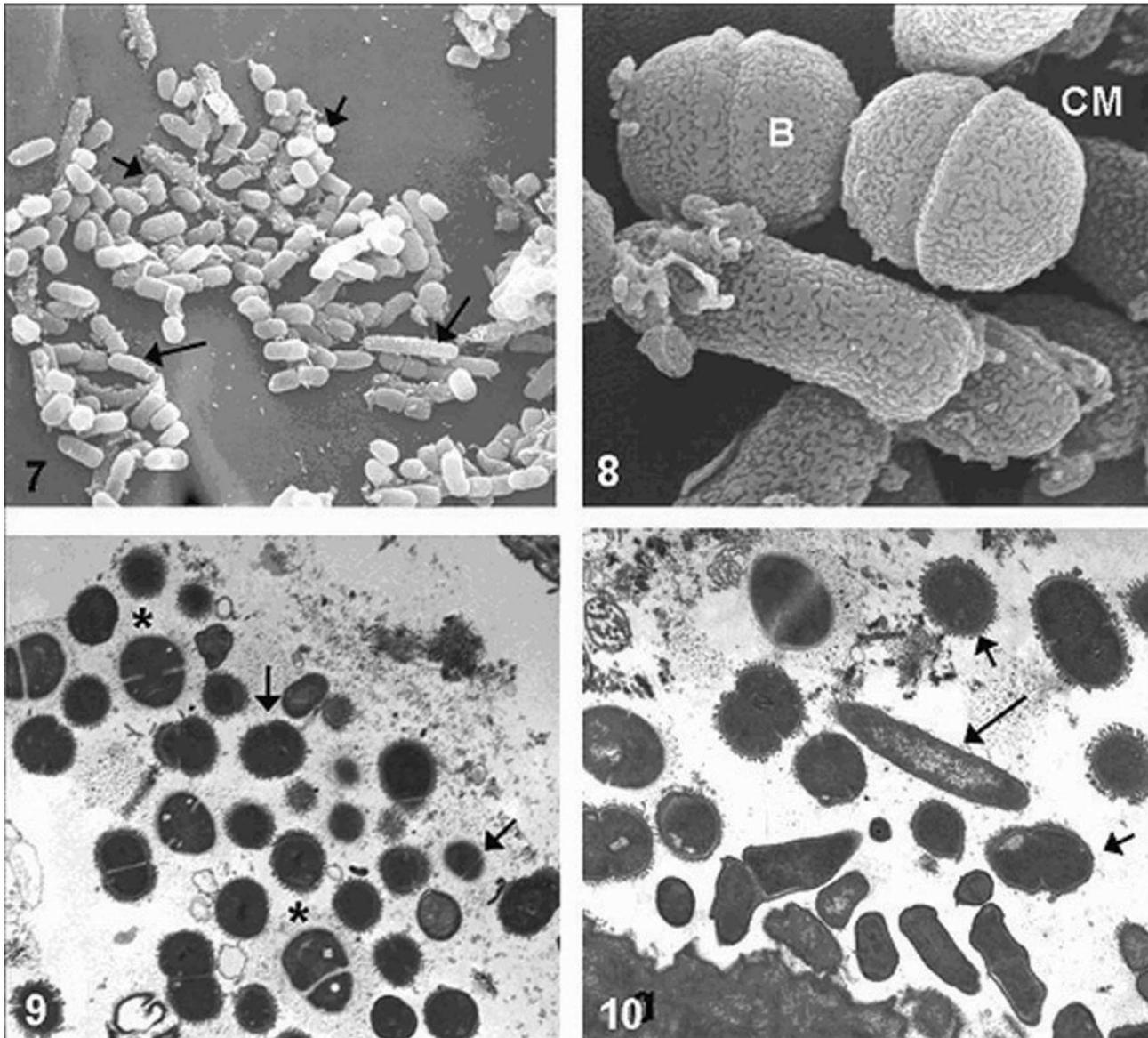


Fig.7 HRSEM images of groupings of coccus (small arrows) and bacillus (large arrows) X 7.000; Fig.8 At high magnification, shows the adhesion between the bacteriae (B) and cell membrane (CM). X 60.000; Fig.9 TEM image revealing the groupings of bacteriae (arrows) and glicocalix (\*). X 12.000; Fig.10 TEM image showing the coccus (small arrow) and bacillus (large arrow) attached by filamentous material to the epithelial cell surface. X 15.000.

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**RESUMEN:** Un día después del nacimiento, la mucosa de la lengua una rata fue examinada por el microscopio electrónico de transmisión y método de ARMEB. Los especímenes fueron fijados mediante un solución Karnovsky y embebido en resina Spurr para microscopía electrónica de transmisión. Para el método ARMEB, las muestras fueron fijadas en tetróxido de osmio 2%, deshidratados en alcohol, secados al punto crítico y recubierto con oro-paladio. Los resultados demostraron que la superficie de la lengua presentaba papilas filiformes y fungiformes cubiertas por numerosas células epiteliales queratinizadas. Las bacterias se unen a las superficies de las micropilas al azar, lo que se demuestra en sus tres dimensiones las imágenes en ARMEB. A gran aumento, las imágenes del microscopio electrónico de transmisión revelan la adhesión de bacterias a la membrana celular por el glicocalix. Las estructuras fibrilares que rodean a las bacterias son claramente visibles.

**PALABRAS CLAVE:** células epiteliales, lengua, rata, coccus, bacillus, ARMEB, MET.

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Accepted: 27-06-2008

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