SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF THE
MAMMARY GLAND MYOEPITHELIAL CELLS OF THE RAT
UNDER NORMAL AND EXPERIMENTAL CONDITIONS

JUNICHI ABE

Department of Obstetrics and Gynecology, Kurume University
School of Medicine, Kurume, 830, Japan

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The stromal surface of the mammary gland myoepithelial cells of rats under normal and experimental conditions, which were treated with HCl-collagenase method introduced by Evan et al. (1976), have been studied by scanning electron microscopy. Treatment of the mammary gland with HCl and collagenase removed almost completely the extracellular materials such as collagens and the basal lamina and revealed the myoepithelial cells enclosing the glandular alveolus. Myoepithelial cells consisting of an elongated cell body and long, bifurcating processes grasped the glandular alveolus in the form of a delicate network. With the application of oxytocin, the myoepithelial cells were involved in the morphological changes suggestive of contraction. They had a row of prominent folds arranged perpendicularly to the long axis of the cell body and its processes.

INTRODUCTION

The mammary gland myoepithelial cells lying on the stromal surface of the glandular alveoli have been studied by a number of investigators (Richardson, 1949; Linzell, 1952, 1955; Takahashi, 1958; Langer and Huhn, 1958; Haguenau, 1958; Bässler et al., 1967; Bässler and Brethfeld, 1968; Murad and Haam, 1968; Bässler, 1970; Hampell, 1970; Radnor, 1972; Sala and Freire, 1974) using light and transmission electron microscopy. The myoepithelial cells lie between the glandular cells and the basal lamina embracing the glandular alveoli in a meshed, basket-like network, and are characterized by their content of tracts of actin-like, parallel filaments, 5 to 6 nm in diameter.

As to the structure of the myoepithelial cell, Richardson (1949) informed the distribution and orientation of it by light microscopy using a silver impregnation method, but his observations were not yet enough to demonstrate the structural details of the myoepithelial cell.

Functionally it may be said that the mammary myoepithelial cells are contractile tissues causing milk ejection in response to oxytocin (Richardson, 1949; Linzell, 1952, 1955; Bässler et al., 1968). By light microscopy, Richardson (1949) and Linzell (1955) found the decrease in volume of the lobules of the mammary gland when oxytocin was applied to. On the other hand, Bässler et al. (1967) revealed morphological changes of the myoepithelial cell with application of oxytocin at the TEM level. Because of the lacking of direct three-dimentional observation by SEM,
it cannot, however, be decided with certainty whether oxytocin makes the myoepithelial cells squeeze the alveoli or not. Moreover, the SEM has not been attempted in the three-dimensional analysis of the myoepithelial cells because they were obscured in intact tissues by extracellular materials, such as collagens and basal lamina, around the alveoli.

Recently, Murakami et al. (1977) have described the SEM findings of the mammary alveoli, but their observations were mainly restricted to the glandular cells of the mammary gland.

In the present study the stromal aspects of alveoli of the rat lactating mammary gland were disclosed by removal of the surrounding connective tissue elements with HCl and collagenase according to the method of Evan et al. (1976) and viewed with SEM. The purpose of this paper is to present the fine surface structures of the myoepithelial cells in rat under normal and contracting conditions and discuss their relationship to the ejection of milk.

MATERIALS AND METHODS

Lactating female rats of Wister strain were used as materials. Animals were divided into two groups by their conditions: normal and experimental. The experimental group were injected in vivo with 1 Unit of oxytocin through the left cardiac ventricle 30 to 40 sec before sacrifice. All the animals were anesthetized with ether and killed by perfusion through the left cardiac ventricle with a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4).

The mammary glands were rapidly removed and immersed in the same fixative for additional 3 hrs. Then, the tissues were placed in 8 N, HCl for 60 minutes at 60°C, rinsed several times in phosphate buffer (pH 6.8), and immersed in phosphate buffered collagenase, pH 6.8 (Worthington, type II) at a concentration of 10 mg/10 cc of buffer for 3 to 8 hrs at 37°C. They were postfixed in cacodylate buffered osmium tetroxide (pH 7.4) for 2 hrs, dehydrated in ascending concentrations of acetone, and dried by the critical point drying method using liquid CO₂. After coating with gold by the ion sputtered method, the specimens were viewed in a Hitachi field emission scanning electron microscope (HFS-II).

RESULTS AND DISCUSSION

I. Normal Group (lactating rats)

In untreated tissues, the alveoli of lactating mammary glands were concealed with the extracellular materials such as a coarse network of collagen fibrils and the coat of basal lamina and were not viewed in SEM as shown in Fig. 1. Treatment of the tissue with HCl and collagenase effectively removed both the collagen fibrils and the basal lamina and exposed the stromal surface of the alveoli which appeared generally flat.

Low magnification SEM image of the basal surface of the glandular alveoli revealed clearly the existence of the myoepithelial cells with long, extending cytoplasmic processes forming a delicate network around the alveoli (Fig. 2). Under light microscopy, the myoepithelial cells of the mammary gland were identified by silver impregnation method (Richardson, 1949) or Gomori's method (Bassler and Brethfeld, 1948). In the present study, the ultrastructural details of the myoepithelial
cells were observed three-dimensionally by SEM. The myoepithelial cell consisted of the elongated cell body slightly bulged due to localization of the nucleus and long, bifurcating processes with basket-like profiles (Fig. 3). The cell bodies of the myoepithelial cells ranged from 20 to 30 μ in length and 3 to 5 μ in width, though the distinction between the cell body and its processes was not necessarily clear. The surface of the myoepithelial cell was relatively flat and smooth, the myoepithelial cell itself lying on the glandular cells or along their boundary. One alveolus was generally embraced by 3 to 4 myoepithelial cells with their processes meeting end-to-end or end-to-side one another (Fig. 4). Adjacent processes appeared to be contiguous with each other to form a delicate network. They were occasionally overlapped. The mammary myoepithelial cells were very long, more extensive and their network was also more dense and delicate than those in the submandibular glands observed by SEM (Nagato 1978; Hamasaki, 1978). Capillary networks were observed along the furrows separating the individual alveoli. Pericytes were seen frequently on the capillary walls (Fig. 4).

Occasionally, the alveoli rugged in appearance were also shown, whose details will be mentioned in the following experimental group.

II. Experimental Group (lactating rats injected with oxytocin)

In lactating rats injected with oxytocin, the mammary gland showed drastic changes in morphology in comparison with the normal mammary gland. Most of all alveoli were rugged in aspect and diminished in volume presumably due to contraction of myoepithelial cells. There was found a wide space between the capillary network and the alveolar wall (Fig. 5, 6).

The myoepithelial cell had a row of prominent folds arranged perpendicularly to the long axis of cell body and processes (Fig. 7, 8). Where there were folds the myoepithelial cell squeezed the basal surface of the glandular cells so deeply that it was more protruded into outside compared with other places. Therefore a cell body and its processes were diminished in length. Bassler et al. (1967) described the TEM image suggestive of the contraction of the mammary myoepithelial cell in oxytocin-treated rats as seen in this study, and stated that at the time of contraction the myoepithelial cell made a row of prominent folds arranged perpendicularly to the long axis of cell body and processes, squeezing the glandular cell into protrusion. Fay and Delise (1973) also observed such a row of prominent folds in the case of contraction of isolated smooth muscle cells by using SEM and TEM. In conclusion, it may be assumed that such a finding of myoepithelial cell as given in Fig. 8 might have been an image of contraction.

The present study provides a strong circumstantial evidence for the view that the mammary myoepithelial cell is a contractile tissue, which contracts in response to oxytocin squeezing the alveoli. The alveoli are diminished in size and the milk accumulated there might be pushed out into the duct. In short, the myoepithelial cells play an important role in milk ejection.

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REFERENCES


**Fig. 1.** Scanning electron micrograph of the mammary alveoli without HCl and collagenase digestion. Their stromal surface is obscured by collagens and basal lamina. ($\times 1,370$).

**Fig. 2.** Low power scanning electron micrograph of the stromal surface of the mammary gland treated with HCl and collagenase. A delicate network of myoepithelial cells lying on the surface of alvols. Capillary network (arrow) is also seen around the alveoli. ($\times 1,100$).
Fig. 3. Higher magnification scanning electron micrograph of the myoepithelial cell. The myoepithelial cell consists of an elongated cell body and long, bifurcating processes. (× 6,200).

Fig. 4. The processes of the myoepithelial cell meet end-to-end (arrow) or end-to-side (double arrows) one another. The myoepithelial cells lie on the glandular cells or along their boundary. A pericyte (P) is shown at the bifurcation of a capillary. (× 1,900).
Fig. 5. The myoepithelial cells squeeze the alveoli so deeply that the individual glandular cells protrude into outside. The alveoli are rugged in appearance. ($\times 1,500$).

Fig. 6. An alveolus diminished in size. A wide space is seen between the capillary and alveolar wall which probably results from squeeging of alveoli. ($\times 3,600$).
Fig. 7. Morphological change of myoepithelial cell at the stage of contraction. The myoepithelial cell has a row of prominent folds arranged perpendicularly to the long axis of cell body and processes. ($\times$ 3,000).

Fig. 8. Higher magnification of the myoepithelial cells as shown in Fig. 7. Note a prominent protrusion of the glandular cells into outside. ($\times$ 7,000).