Scanning Electron Microscopic Study of the Mitotic Figures in Cultivated Cells*

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Summary. Two cell lines established from human stomach cancers were used in this experiment. After culture by the coverslip method, cells were fixed and coated with carbon and gold to be observed under the scanning electron microscope.

1. When the cells began to grow in a monolayer after the subculture, the boundaries between interphasic cells were indistinguishable. The nuclei in the interphasic cells were thinner than the surrounding cytoplasm and appeared concave. However, the nucleoli were prominent in the surrounding karyoplasm and appeared convex.

2. The cell which entered the mitotic stage rose spherically among the flat interphasic cells and stretched out many fine cytoplasmic processes (0.6 µ thick) in all directions.

3. In the telophase, two daughter cells were connected with a bundle of many fine cytoplasmic processes.

4. In general, the mitotic cell surface was covered with a fine granular structure which was different in appearance from a microvilli-like structure. This structure is supposed to be due to the bubbling of mitotic cells observed by time lapse cinematography.

The recent development of scanning electron microscopy provides an opportunity to study the surface structure of various tissues and cells. Most of the studies which have hitherto been reported concern hard tissues or soft tissues of man or animals obtained in vivo (cf. Fujita, Tokunaga and Inoue, 1971). On the other hand, very few papers are available which make use of cultivated cells in vitro for scanning electron microscopic study up to the present time (Boydé et al., 1968; Boydé, Grainger and James, 1969; Fiskin and Melnykovych, 1971; Dalen, Schlaffer and Mamoon, 1971). The cultivated cells should be very useful material for the observation of the cellular surface of a single cell. This paper deals with scanning electron microscopic observations of the mitotic figures in the cultivated cells of two established cell lines.

Materials and Methods

The two cell lines used in this experiment have been established from a human stomach cancer (SC6809) and a human metastatic liver tumor from a stomach cancer (SCLvM6901). The detailed process of the establishment will be reported elsewhere (Nawa, 1972). These cell lines are subcultivated two times every week by means of ethylenediamine tetraacetic acid (EDTA) solution. The medium used for the culture consisted of Eagle’s MEM (produced by Nissan, Tokyo) and 10% bovine serum. After EDTA treatment, cell suspensions were injected into culture bottles containing coverslips. When the confluent sheet of cells were obtained after two days incubation at 37°C, the coverslips on which the cells adhered were fixed for one hour.

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The fixative mostly used was 2.5% glutaraldehyde buffered with 0.1 M cacodylate at pH 7.3. The fixed cells were then dehydrated in a graded series of acetone and finally air-dried. The dried cells were coated with thin layers of carbon and gold in a vacuum evaporator. The specimens were examined in a scanning electron microscope, JEM-U3, Japan Electron Optics Laboratory Co., Ltd.

**Observations and Discussions**

When the cells began to grow in a monolayer after the subculture, the boundaries between adjoining cells were indistinguishable (Fig. 1). It is supposed that this is due to the arrangement of the confluent cells which are tightly in contact with each other. As shown in Figures 1 and 2, interphasic cells ordinarily had a few nucleoli which were easily recognizable by their prominency in the surrounding karyoplasm. On the other hand, the nuclei in interphasic cells were thinner than the surrounding cytoplasm and appeared concave. This difference may be possibly due to the total water content among the nucleus, nucleolus and cell body (Boyde et al., 1968).

Among the flat interphasic cells, mitotic cells which had entered into prophasic stage appeared hemispherical (Fig. 1, 2). These mitotic cells stretched out many fine cytoplasmic processes which are 0.6-1.0 μ thick and 5-8 μ long in all directions. Figure 3 shows a protelophasic cell which had a division furrow in the cytoplasmic center. Figure 4 shows a succeeding stage. Namely, the cytoplasmic division went on to a more advanced stage, and then the two daughter cells stretched out to right and left. Figures 5 and 6 show further stages of cytoplasmic division. Figure 5 shows

![Fig. 1. A scanning electron micrograph of SCLvM6901 cells in interphase. The nuclei are concave, while the nucleoli appear prominently raised in the nuclei. Mitotic cells appear hemispherical among the flat interphasic cells. ×900](image-url)
Fig. 2. A scanning electron micrograph of SCLvM6901 cells showing two mitotic cells among the flat interphasic cells. The mitotic cells stretch out many fine cytoplasmic processes in all directions. $\times 1,800$

Fig. 3. The pro-telophase stage from SCLvM6901 cells which have a division furrow at the center of the cytoplasm. $\times 3,000$
Fig. 4. An SCLvM6901 cell in a more advanced stage than in Figure 3. The cytoplasmic division is proceeding. ×3,000

Fig. 5. A scanning electron micrograph of two daughter cells from SCLvM6901 cells which are connected with a bundle of many fine cytoplasmic processes. ×2,400
a later telophasic figure by which the two daughter cells were connected with a bundle of many fine cytoplasmic processes. In Figure 6 the mitotic figure proceeded to the final stage from Figure 5. The two daughter cells were connected only with a single thin process of cytoplasm which would further be torn off.

In general, the cultivated cells which entered into the mitotic stage raised rapidly taking a hemispherical form, and then transformed rapidly in the telophase showing violent bubbling of the cytoplasm, when they were observed by time lapse cinematography (Nawa, 1968). By observation with an interference microscope, the colar balance of cells was changed when the cells went into mitosis showing a change in their optical density (Nagata, 1970). This phenomenon seemed to reflect the changes in the thickness and surface structure of mitotic cells observed by scanning electron microscopy in this paper. Figure 7 shows the metaphasic cells whose cytoplasmic processes stretched out in all directions. Moreover, the surface of these cells was covered with fine granular structures. Figure 8 shows a fine granular structure on the mitotic cell surface at a higher magnification. Figure 9 shows the microvilli-like structure found on some mitotic cells. The structure shown in Figure 9 markedly differs from that of Figure 8. The structure of Figure 9 resembles the microvilli observed in ascites tumor cells by Hayes et al. (1966). Size measurement indicated the diameter of the microvilli-like structure in Figure 9 to be about 0.15 μ, while that of the granular structure in Figure 8 to be 0.6 μ. On the other hand, the frequencies of these two structures were different, i.e., the latter was more frequently observed than the former in mitotic cells. These data seem to permit a presumption that the

Fig. 6. Two daughter cells of SCLvM6901 cells in a more advanced stage than in Figure 5. The two daughter cells are connected only with a single cytoplasmic process. ×3,000
Fig. 7. A scanning electron micrograph of SC6809 cells. The cell surface in the mitotic stage is covered with a fine granular structure. ×2,000

Fig. 8. A high power view of an SCLvM6901 cell in mitosis. Note that the cell surface is covered with a fine granular structure. ×5,000
former may be the microvilli while the latter be the bubbling which can be observed in time lapse cinematography. Further study is needed to examine the validity of this assumption.

From the results obtained in the present study, it has been clarified that the cells which entered the mitotic stage took a hemispherical form stretching out many cytoplasmic processes in all directions, then cytoplasmic constriction appeared between the two daughter cells which were later connected with a bundle of many fine cytoplasmic processes, and finally a thin filamentous cytoplasmic process which connected the two daughter cells was torn off, thus separating the two daughter cells.

Examining the literature treating the scanning electron microscopy of cultivated cells, very few papers are available up to the present time as far as the present authors know. Boyde et al. (1968) observed fibroblasts, neuroglial cells and neurons in cultures from chick embryo spinal cord. Boyde et al. (1969) observed fibroblasts from chick embryo hearts, Fisken and Melnykovych (1971) HeLa cells were treated with prednisolone, while Dalen et al. (1971), the ciliated ependymal cells from the cerebellum of newborn rats. However, no paper has referred to the mitotic figures of these cells. Therefore, this paper is believed to be the first which describes mitotic figures observed by scanning electron microscopy.

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ヒト胃癌細胞から樹立した2つの細胞系（SC6809, SCLvM6901）を材料にして、その分裂像を走査電子顕微鏡で観察した。

1. 単層になって増殖し、分裂間期にある細胞は扁平で、隣接する細胞と細胞との境界は不明である。核は周囲の細胞質より陥凹して見える。しかし核小体は突出している。

2. 分裂期に入ると扁平な細胞が球形に盛り上がり、細胞表面には微鉤毛と思われる細長い細胞質の突起（径0.15μm）が放射状に突出している像と、微鉤毛より太く長い突起（径0.6μm）が突出している像が見られる。

3. 細胞分裂終期において、2個の娘細胞は微細な細胞質の突起の束によって結合されている。

4. 盛り上がった分裂期の細胞表面は微細な顆粒状の構造を呈する。この構造は通常みられる細胞表面の微鉤毛にくらべて短かく太い（径0.6μm）。これは培養細胞を位相差顕微鏡映画撮影で観察するさい認められるbubblingに相当するとと思われる。

References


