Scanning Microscopy

Volume 5 | Number 2 Article 28

3-15-1991

Growing and In-Situ Processing of Cells on BEEM Capsule Caps for Scanning Electron Microscopy

Eugene F. Bernard
Walter Reed Army Institute of Research

Tatsuo Hase
Walter Reed Army Institute of Research

Scanning Microscopy, Vol. 5, No. 2, 1991 (Pages 595-599) 0891-7035/91$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

GROWING AND IN-SITU PROCESSING OF CELLS ON BEEM CAPSULE CAPS FOR SCANNING ELECTRON MICROSCOPY

Eugene F. Bernard and Tatsuo Hase*

Department of Ultrastructural Pathology, Division of Pathology
Walter Reed Army Institute of Research
Washington, DC 20307

(Received for publication September 27, 1990, and in revised form March 15, 1991)

Abstract

A simple technique for processing cells grown on BEEM capsule caps for scanning electron microscopic (SEM) observation is described. The cells adhere to a substratum on the inner surface of caps coated with either an egg white-polyvinylpyrrolidone solution or a polylysine solution in the case of eukaryotic cells and an agar solution in the case of bacteria. The adhering cells are cultured and then processed in situ for SEM observation. Electron micrographs revealed details of the shape of the cells growing on the surface of the substrate. The technique seems to be particularly well suited for observing sequential morphological changes of cells, bacteria, and protozoa cultured under a specified experimental condition as well as of interactions between cells and parasitic microorganisms.

Key words: AIDS virus, BEEM capsule cap, cell culture, colibacilli, erythrocyte sickling, gonococci, human polymorphonuclear leukocytes, in-situ processing, Leishmania, mouse splenic cells, Trypanosoma

*Address for correspondence:
Tatsuo Hase
Department of Ultrastractural Pathology
Division of Pathology
Walter Reed Army Institute of Research
Washington, DC 20307
Telephone Number: 202 576 2307
FAX Number: 202 576 3114

Introduction

One disadvantage of scanning electron microscopy (SEM) is that living cells cannot be directly observed under the microscope. A growth pattern of cells in culture, their interactions with other microorganisms, and sequential changes that may occur at different time intervals during the culture are difficult to study. Previously, we reported a simple transmission electron microscopic (TEM) technique for growing cells on BEEM capsule caps (size 00, BEEM Inc., Bronx, NY) and fixing and embedding these cells in situ at different time intervals (Asafo-Adjei et al., 1987). This technique can also be applied for SEM, and it can provide a useful means for observing sequential changes of cellular sizes, shapes, and appearances in a specified experimental condition. This paper describes a technique for in-situ processing of cells grown on BEEM capsule caps for SEM and reports some of the results that were obtained with the technique.

Materials and Methods

Growing cells

One hundred BEEM capsule caps were placed in a 2,000-ml beaker with 1,000 ml acidic water (3 drops of 70% nitric acid) and boiled for 1 hour. The caps were then rinsed thoroughly with distilled water and coated with an egg white-polyvinylpyrrolidone solution (Brigati et al., 1983) as described previously (Asafo-Adjei et al., 1987). In some cases, the caps were coated with a poly-D-lysine solution (50 μg/ml, MW 70,000-150,000, Sigma Chemical Co., St. Louis, MO) by spreading 50 μl of the solution over the inside of each cap and drying the solution in an oven at 65°C for 2 hours. For the culture of *E. coli*, the insides of the caps were coated with a 5x diluted tryptic soy agar solution in the same manner as they were coated by the polylysine solution. The coated caps were stored at 4°C until use. Prior to cell growth, the caps were sterilized by exposure to a ultra violet (UV) germicidal lamp for 30 minutes. For a cell or bacterial culture, a drop of cell or bacterial suspension was placed in the center of each

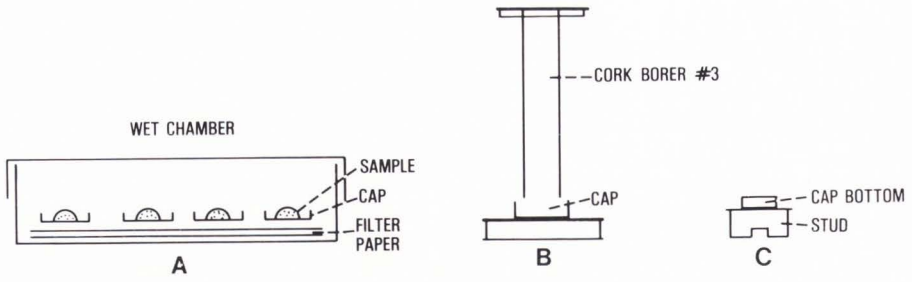

Figure 1. a. Arrangement of samples on BEEM capsule caps in a wet chamber for incubation. **b**. Removal of the edge of a cap by a #3 cork borer before mounting. **c**. Mounting of the flat part of a cap on the stud.

cap, and the cap was placed in an appropriate moist chamber for incubation as shown in Fig. 1a. The moist chamber was placed in an incubator for culture for a selected time. For a sequential study, a large number of samples could be prepared and handled without any difficulty. Mammalian cells usually adhered to the surface of the cap within 1 hour, and bacteria within 2 hours.

In-situ processing

The eukaryotic cells or bacteria adsorbed on the surface of the cap were washed once by dipping the cap into phosphate-buffered saline (PBS) and then fixed by placing the cap in 4F1G fixative (NaH_2PO_4 H_2O, 11.6 gm; NaOH, 2.7 gm; 37-40% formaldehyde, 100 ml; 50% glutaraldehyde, 20 ml; to make up to 1,000 ml with water: McDowell and Trump, 1976) for 1 hour at room temperature. The samples were then washed with 0.1 M cacodylate buffer (pH 7.4), postfixed in 1 percent cacodylate-buffered osmium tetroxide, and dehydrated in a series of graded ethanol-water solutions ending in absolute ethanol. The samples were dried by a critical point apparatus (Samdri, Tousimis Research Corporation, Rockville, MD), using liquid carbon dioxide as the transition fluid. The rim of the cap was removed by using a #3 cork borer (Fig. 1b). The base of the cap was placed on the stud using a sticky tab (Ernest F. Fullam, Inc., Latham, NY) as shown in Fig. 1c, and coated with gold/palladium in an automatic sputter-coating apparatus (Samsputter-2a, Tousimis Research Corporation). The samples were examined with a Hitachi S-450 scanning electron microscope operated at 20 kV.

Results and Discussion

We examined a variety of different types of specimens by the present technique. When a drop of blood obtained from a sickle-cell-anemia patient was placed with a drop of 2% sodium metabisulfite solution on a BEEM capsule cap coated with egg white-polyvinylpyrrolidone and was incubated for 30 minutes at room temperature, the erythrocytes that adhered to the substratum showed bizarre shapes, indicating ocurrence of sickling (Fig. 2a).

In Fig. 2b, a small piece of perfused mouse spleen was immersed in a drop of culture medium (medium 199 with 10% fetal bovine serum) placed on a BEEM capsule cap coated with egg white-polyvinylpyrrolidone and incubated for 1 hour at 37°C. At the end of incubation, the splenic piece was removed, and a drop of promastigote suspension (70% Schneider's medium and 30% fetal bovine serum) of *Leishmania mexicana mexicana*, WR-224, was added, and the mixture was incubated for another hour at 37°C. The scanning electron micrograph shows promastigotes (arrowheads) attaching to wandering splenic cells.

In Fig. 2c, a drop of mouse blood infected with *Trypanosoma brucei* was placed on a BEEM capsule cap coated with egg white-polyvinylpyrrolidone and incubated for 1 hour at 37°C. The scanning electron micrograph shows a trypanosome (arrowhead) among red blood cells.

In Fig. 2d, a drop of human polymorphonuclear leukocyte (PMN) suspension (RPMI 1640, Gibco, containing 10% fetal bovine serum, 4 μg/ml glutamine, and antibiotics) prepared by percoll fractionation (Harbeck et al., 1982) was placed on a BEEM capsule cap coated with egg white-polyvinylpyrrolidone and incubated at 37°C; 1 hour later, a drop of gonococcal (*Neisseria gonorrhoeae*) suspension was added in the ratio of 20 bacteria per cell and incubated for an additional 5 minutes. The scanning electron micrograph shows gonococci (arrowheads) in the process of phagocytosis by polymorphonuclear leukocytes adhered to the substratum.

In Fig. 2e and f, a drop of H-9 human lymphoblastoid cell suspension (RPMI 1640, Gibco, containing 10% fetal bovine serum, 4 μg/ml glutamine, and antibiotics), was placed on a BEEM capsule cap coated with polylysine and incubated for 1 hour at 37°C; then, a drop of an inoculum containing AIDS virus (HTLV IIIB) was added. After 4 days of culture, the electron micrographs show numerous budding AIDS virus particles (arrowheads) on the surfaces of H-9 syncytial cells.

In Fig. 3, a drop of pen-assay broth in which *Escherichia coli* had been growing for 24 hours was placed on each of BEEM capsule caps coated with tryptic soy agar and incubated for 2 hours at room temperature. At the end of incubation, the bacteria were washed once with PBS and treated with the 12.5% sucrose solution containing lysozyme (1 mg/ml) and EDTA (30 mg/ml) for 15 minutes at 0°C and then 5 minutes at 37°C by flooding the solution to the rim. The bacteria were then washed with PBS and covered with fresh pen-assay broth for further incubation for selected time intervals. The scanning electron micrographs show untreated colibacilli (Fig. 3a), rounded protoplasts at 1 hour after the treatment (Fig. 3b), mixture of rounded

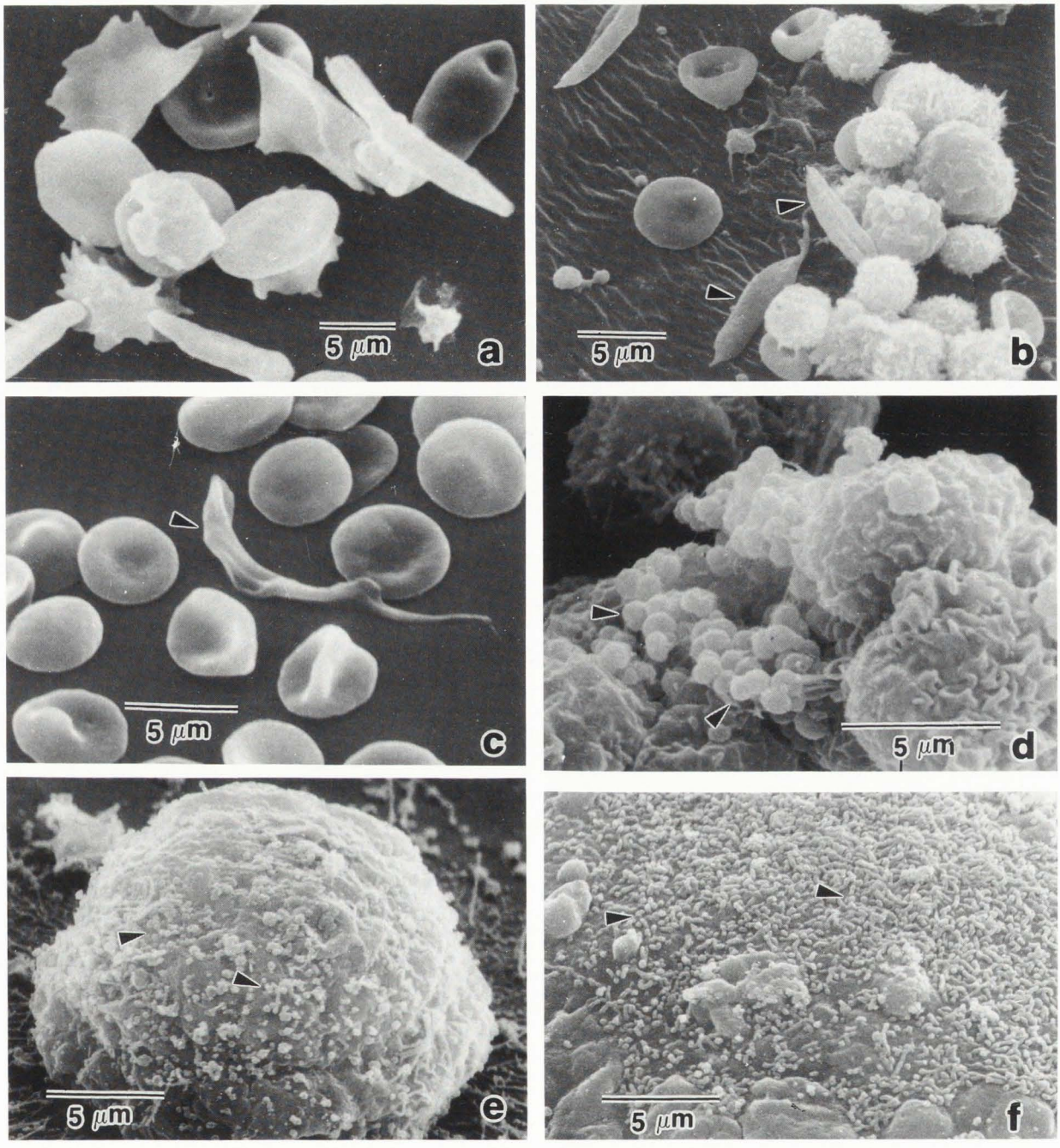

Figure 2. a. Erythrocytes from a sickle-cell-disease patient showing variable, bizarre shapes as a result of sickling. **b**. Promastigotes (arrows) of *Leishmania mexicana mexicana* attaching to mouse splenic wandering cells. **c**. A *Trypanosoma brucei* (arrow) in the blood of an infected mouse. **d**. Gonococci (arrows) attaching to human polymorphonuclear leukocytes. **e and f**. AIDS virus (arrows) budding from H-9 syncytial cells.

protoplasts and reverting rod-form colibacilli at 24 hours after the treatment (Fig. 3c), and return to rod-formed colibacilli at 48 hours after the treatment (Fig. 3d).

Admittedly, for observation of cultured cells under the light microscope, the coated BEEM capsule caps are less transparent and therefore less satisfactory than the coated coverslips that are frequently used for similar purposes. However, when electron microscopic study is

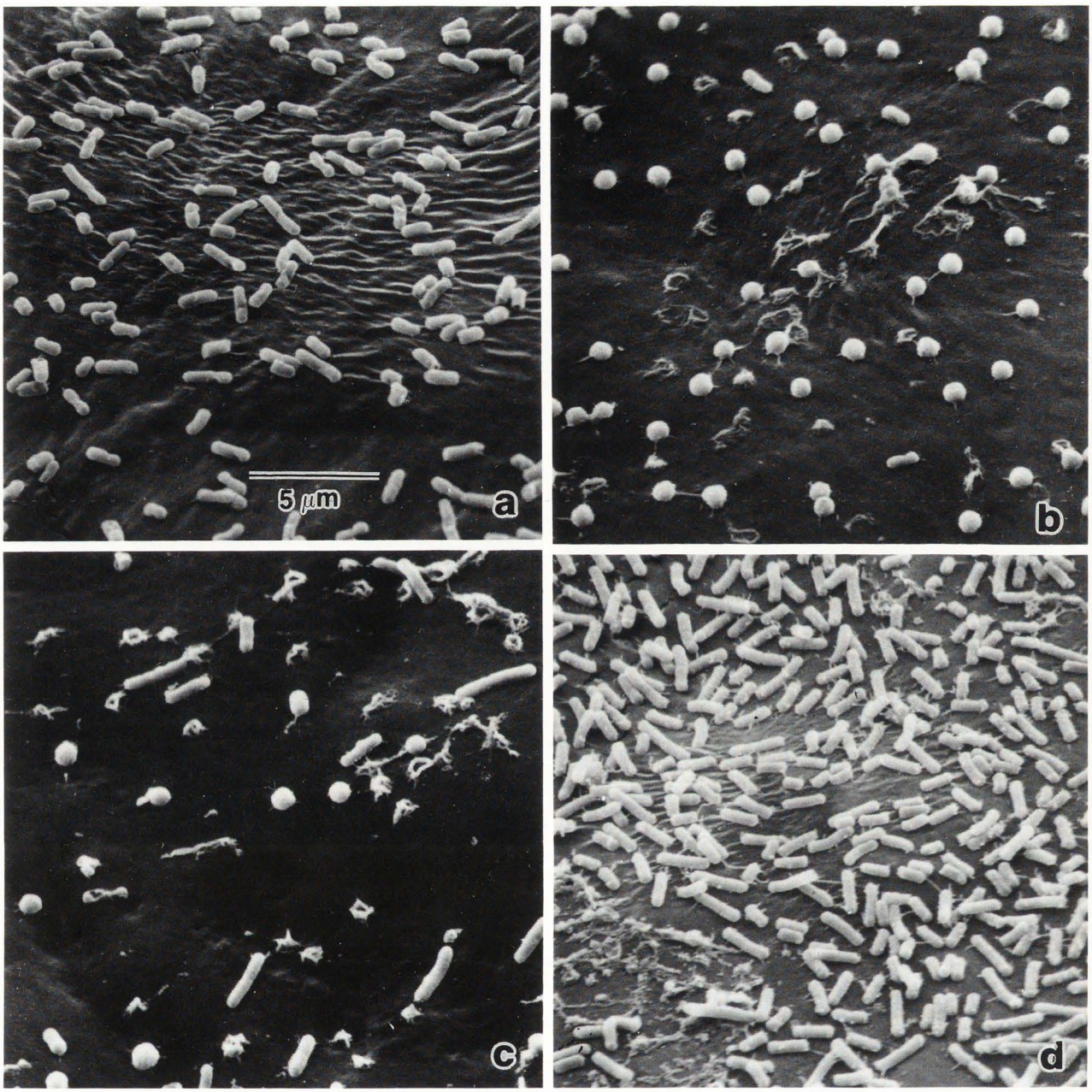

Figure 3. Change in shape of *E. coli* brought about by a lysozyme treatment. **a**. Rod-form bacteria before the treatment. **b**. Round protoplasts 1 hour after the treatment. **c**. Reversion of protoplasts to rod-forms 24 hours after the treatment. **d**. Multiplication of rod-forms 49 hours after the treatment.

solely intended, the caps are much easier to handle in large numbers than the coverslips, without any worry of breakage. Moreover, we can process some caps for TEM as described previously (Asafo-Adjei et al., 1987) and others for SEM as described here. This enables us to examine a sample grown in a same condition by both TEM and SEM for comparison. The surface of the cap is coated smoothly by any of the solutions used in this study and provided a good background for observing cultured cells by SEM. Occasionally, fine rippled surfaces appeared locally, but they did not at all interfere with observation of cultured cells. The BEEM capsule caps and sticky tabs did not lead to charging problems. The present method is suited for culture of a large number of samples of eukaryotic cells or bacteria individually. Cells or bacteria can be treated with various agents or

interacted with other types of cells or bacteria during the culture, and resulting changes can be observed chronologically by SEM by removing a certain number of caps at a time from the culture chamber.

Acknowledgement

The authors are grateful to the following investigators for generously supplying the samples: Dr. S. Formal for the *E. coli* suspension, Dr. B. D. Hansen for the Leishmanial suspension, Dr. J. Ribas for the AIDS virus-infected H-9 cell preparation, and Dr. H. Schneider for the gonococcal suspension.

References

Asafo-Adjei E, Bernard EF, Hase T (1987) Growing and embedding monolayer cells in situ in Beem capsule caps for light and electron microscopy. J. Electron Microsc. Tchn. 5, 367-372.

Brigati DJ, Myerson D, Leary JJ, Spalholz B, Travis SZ, Fong CKY, Hsiung GD, Ward DC (1983) Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. Virology 126, 32-50.

Harbeck RJ, Hoffman AA, Redecker S, Biundo T, Kurnick J (1982) The isolation and functional activity of polymorphonuclear leukocytes and lymphocytes separated from whole blood on a single percoll density gradient. Clin. Immunol. Immunopathol. 23, 682-690.

McDowell EM, Trump BF (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100, 405-414.

Discussion with Reviewers

S. L. Goodman: When cutting off the rim of the cap does the disk ever remain inside the cork borer? How is the disk removed without damaging the specimen surface? We find that when examining specimens adherent to plastic supports that a line of conductive paste from the plastic support to the stub is necessary to ensure proper grounding. How thick is your Au/Pd coat?

Authors: Yes, the disk remains inside the cork borer. We can take advantage of this by placing the end of the cork borer on top of the stud covered with a sticky tab and pushing the edge of the disk by an applicator stick so that the disk is pressed directly on to the sticky tab. We sputter Au/Pd at 50 millitorr and 40 milliamps for 30 seconds that will give a coating of 100 Angstroms on a smooth surface. For monolayer cell specimens, we have not experienced any charging problems using the above procedure.