Electron microscopy technology of reticulocytes after sorting with magnetic beads

The Cell Analysis Center – Scientific Bulletin Part 2

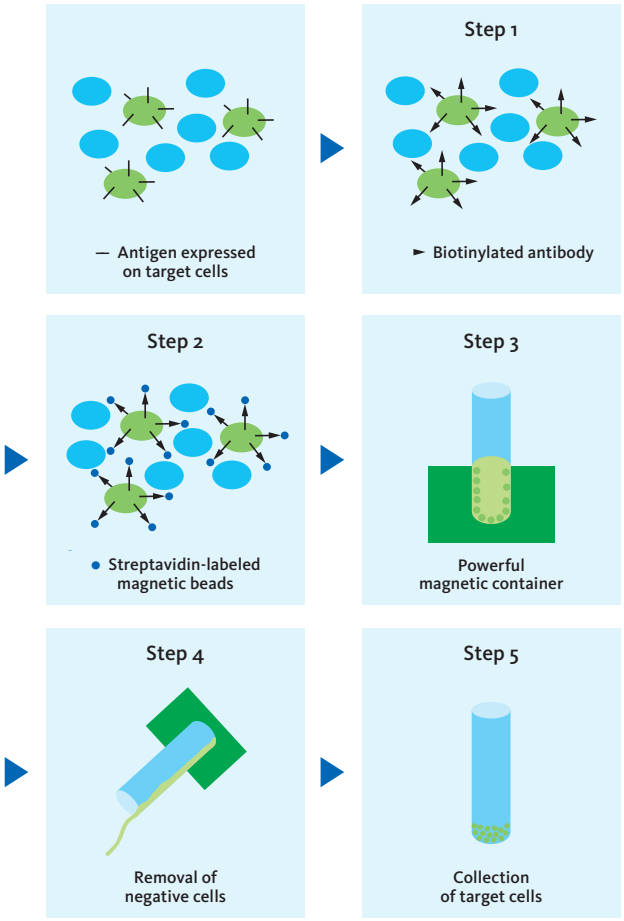
For efficient analysis of cells, sorting of the target cells is crucial. When immature erythrocytes (reticulocytes[1]) are analysed, cell sorting technology plays an important role in detailed analysis of these cells as reticulocytes account for about 1% of all erythrocytes. Electron microscopy is the only means available for direct observation of the ultramicrostructure[2] of cells and tissues. However, specific pretreatment of samples is needed for some analyses (localisation[3] of cell protein, etc.) using an electron microscope. This bulletin presents the technology for cell sorting and subsequent observation of ultramicrostructure of cells.

Cell sorting technology

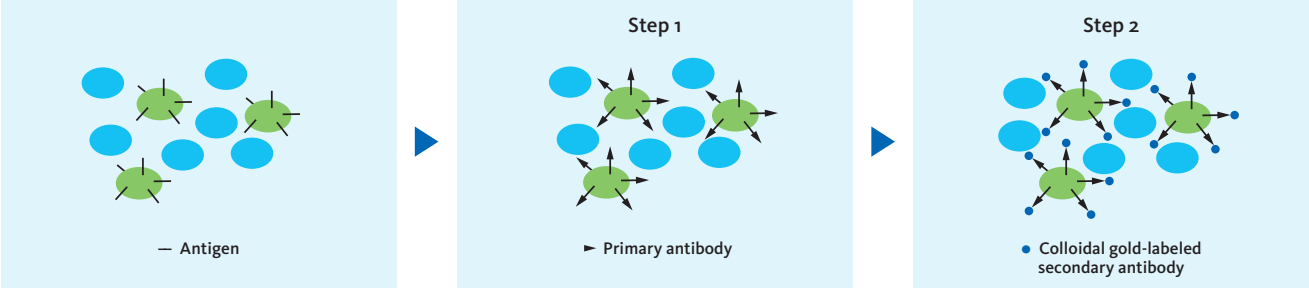
Cell sorting technology pertains the isolation of characteristic parts of cells (e.g. cell surface antigen) labeled with various methods. Major techniques available for cell separation are FACS (Fluorescence Activated Cell Sorting) and MACS (Magnetic Cell Sorting). FACS is a method in which cells are electrically sorted making use of the principle of flow cytometry[4]. MACS is a method in which cells are sorted with magnetic power after magnetic beads are attached to cell surface. The advantage of MACS is that there is no need for special manipulation or skill. MACS is outlined beside [1].

What is MACS?

First, the target cells are incubated with a biotinylated antibody[5] for a surface antigen (Step 1). Then, the cells are incubated with streptavidin[6]-labeled magnetic beads (Step 2) and set in a powerful magnetic container (Step 3). The cells to which magnetic beads have attached will adhere to the wall of the tube by magnetic force, while negative (beads-free) cells float within the solution. The negative cells are removed by decantation[7] (Step 4). The tube is taken out of the powerful magnetic container, and the target cells are harvested (Step 5).



What is colloidal gold labeling technique?



Colloidal gold[8] labeling is a technique of immunoelectron microscopy[9] by which antigens in cells and tissues are labeled with gold particles for subsequent ultramicrostructural observation and immunohistochemical[10] examination under an electron microscope[11], using antigen-antibody reactions[12]. If an electron beam is applied to colloidal gold particles, many reflected electrons[13] are released and the particles are visible as bright spots under a scanning electron microscope[14]. Under a transmission electron microscope[15], the particles are visible as black spots because electrons scatter[16]. The greatest advantage of immunoelectron microscopy is that it allows observation of ultramicrostructure and localisation of target substances (protein, etc.) at a time.

Colloidal gold labeling is performed in the following steps: First, the antigen expressed on the target cells is hybridized to a primary antibody (Step 1). The cells are then hybridized with a colloidal-gold labeled secondary antibody which has a high affinity to the primary antibody (Step 2). The sample is then observed under an electron microscope. For comparison with the previously outlined technique of purification using magnetic beads (MACS), the example of this technique shown above uses a biotinylated antibody as the primary antibody and streptavidin-labeled colloidal gold as the second step.

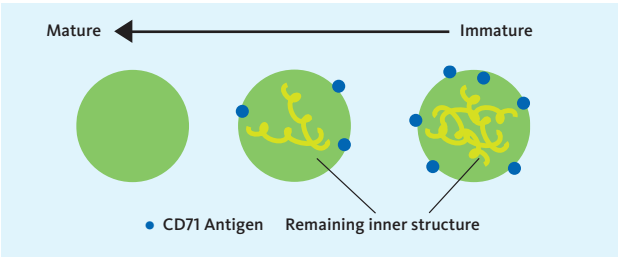
Observation of the ultramicrostructure of reticulocytes under an electron microscope after sorting with magnetic beads

We attempted to observe the reticulocytes attached with magnetic beads after cell sorting by MACS to perform observation of the ultramicrostructure of CD71-positive reticulocytes and localisation of this antigen[17] efficiently. In comparison, immunoelectron microscopy with colloidal gold (colloidal gold labeling) was performed.

When magnetic beads are observed under an electron microscope after MACS, we can simply perform sorting of reticulocytes and determine the location of target substances.

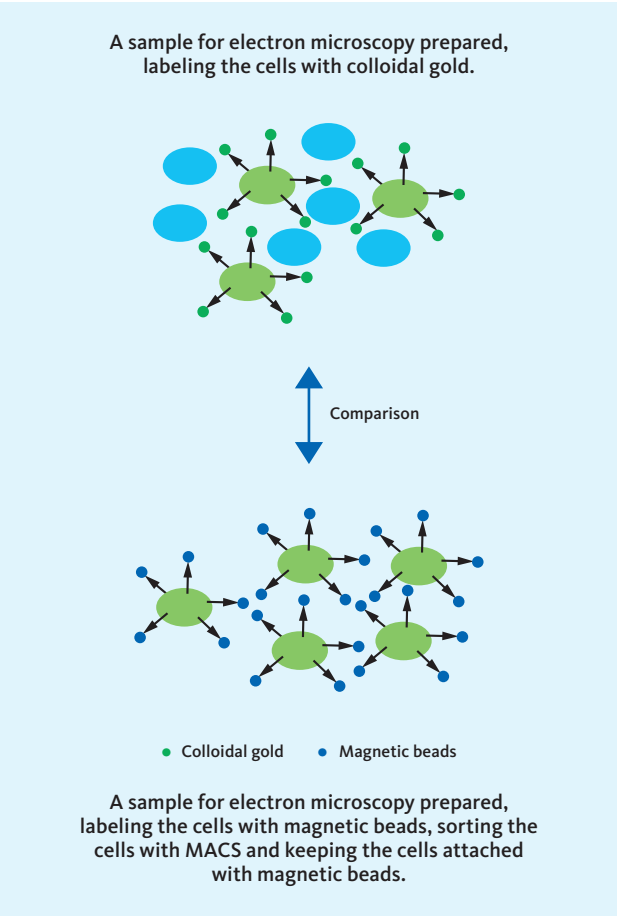
Reticulocytes and CD71 antigen

Reticulocytes are immature erythrocytes found in peripheral blood[18]. The percentage of reticulocytes among all erythrocytes serves as an indicator of anaemia[19] and the haematopoietic capability of bone marrow[20] [2]. Reticulocytes contain some organelles such as ribosomes (with nucleic acids)[21] [3]. Transferrin receptor[22] (which is a membrane-bound protein[23]) on the surface of reticulocytes regulates iron metabolism[24] through binding to transferrin[25] [4]. Both the below-mentioned inner structure and CD71 antigen of reticulocytes disappear as the cells become mature [5].



Comparison between colloidal gold labeling and magnetic beads labeling techniques

Because the diameter of the magnetic beads used for MACS is 10 nm, 10 nm colloidal gold particles were also used for colloidal gold labeling, and the two methods were compared.



Comparison of the images of reticulocytes obtained with two techniques of immunoelectron microscopy

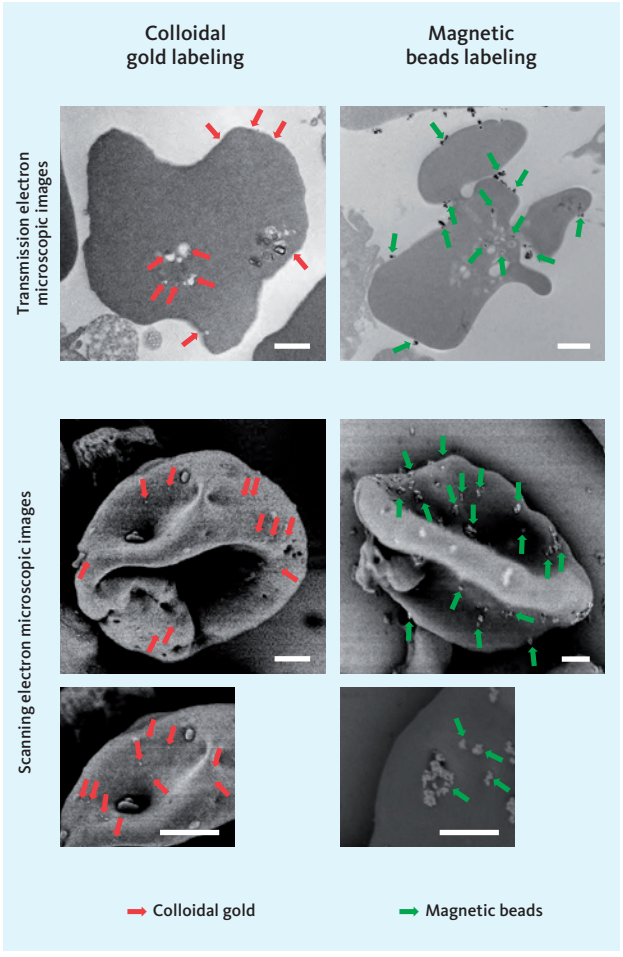
When immunoelectron microscopy is performed using the colloidal gold labeling technique, individual gold particles adhere to the cell surface, allowing detailed observation of CD71-expressed sites. However, colloidal gold (ca. 10 nm) adhering to reticulocytes is difficult to observe at low magnifications (ca. x 1500).

When magnetic beads labeling is employed, the magnetic beads adhering to cell surface form a cluster (joining free magnetic beads by magnetic force). For this reason, the mass of magnetic beads adhering to reticulocytes can be observed instantaneously even at low magnifications, although the detailed distribution of CD71 antigen is slightly less clear than with the colloidal gold labeling technique.

To emphasize the distribution of the target substance during observation with the colloidal gold labeling technique, one possible means is to use colloidal gold with a larger size. However, this sometimes reduces the sensitivity of detection. These problems may be avoided by the magnetic beads labeling technique which allows the use of relatively small metal particles (10 nm in diameter).

Magnetic beads labeling is advantageous as illustrated below when used for electron microscopy. In addition, it allows sorting and concentration of target cells by means of MACS. It is a new technique allowing cell sorting and pretreatment for immuno-electron microscopy at a time.

Both colloidal gold labeling and magnetic beads labeling cause no problem in observation of ultramicrostructure of cells under an electron microscope.



Bar=1μm

Terminology

1 Reticulocyte
Immature erythrocytes just released from bone marrow into blood vessels. About 1% of all erythrocytes are reticulocytes in healthy individuals.

2 Ultramicrostructure
Detailed structure of organisms (cells, tissues, organs, etc.) which are visible only under an electron microscope (not visible under biomicroscopes).

3 Localisation
Location where substances are present.

4 Flow cytometry
Small particles such as cells are dispersed in a fluid, and the fluid is flowed through a small nozzle for optical analysis of individual particles. Sysmex's automated haematology analysers are based on this principle.

5 Biotinylated antibody
Antibodies labeled with biotin (a water-soluble vitamin). Because biotin binds irreversibly to glycoprotein avidin, it is often used as a reagent in research on immune reactions and cell membrane.

6 Streptavidin
A protein binding strongly to biotin.

7 Decantation
A manipulation by which a container is gently inclined to let only the supernatant flow out.

8 Colloidal gold
Gold particles (1–40 nm) used for immunoelectron microscopy. Gold with a high electron density is visible as black particles under a transmission electron microscope and as white particles under a scanning electron microscope.

9 Immunoelectron microscopy
A technique of electron microscopy by which the substances in tissues or cells are observed under an electron microscope through enzymatic reactions or labeling with heavy metals, making use of antigen-antibody reactions. Labeling with a heavy metal (colloidal gold) was employed in this bulletin.

10 Immunohistochemistry
A technique of histological examination by which antigens in tissue specimens are explored using antibodies.

11 Electron microscope
Microscopes allowing high-resolution observation at high magnifications, making use of electron beams whose wavelength (0.01 nm) is shorter than that of the light used for biological microscopes (350–800 nm).

12 Antigen-antibody reactions
Highly specific reactions taking place between antigen and antibody (resembling the relationship between key and keyhole).

13 Reflected electrons
Electrons colliding with the atoms of the sample and rebounding backwards without losing the initial energy during observation under a scanning electron microscope, being used as a signal of the structure of the sample.

14 Scanning electron microscope
A microscope allowing observation of the ultramicrostructure of the cell surface. A special metallic film is formed on the cell surface, and electron beams are applied to it for observation of the cell surface.

15 Transmission electron microscope
A microscope allowing observation of the microstructure inside cells. The cells are made into thin slices (70 nm) and electron beams are applied to them for visualization of electrons.

16 Scatter
Waves (light, etc.) and particles change the direction of their movement through collision or interactions with the target.

17 Antigen
Specific molecules (proteins, etc.) causing immune reactions.

18 Peripheral blood
Blood flowing through blood vessels.

19 Anaemia
A condition characterised by a reduction in erythrocyte count or haemoglobin level in blood.

20 Haemopoietic capability of bone marrow
The capability of bone marrow (an organ producing blood cells) to produce blood.

21 Ribosome
An organelle which receives genetic information from messenger RNA and synthesizes proteins.

22 Transferrin receptor
A membrane-bound protein which binds to transferrin and receives iron.

23 Membrane-bound protein
Proteins with a trans-membranous portion, found on cell surface.

24 Iron metabolism
Conversion of exogenous iron into a form fit for utilization in vivo.

25 Transferrin
A glycoprotein formed in the liver and carrying iron. Molecular weight is 78000.

Reference

[1] Dainiak M. B. et al. Methods in cell separations. Adv Biochem Eng Biotechnol. 2007 Jul. 28.

[2] Bessman J. B. Reticulocytes. Clinical methods third edition. 1990; 156: 735–738.

[3] Baldini M. and Pammacciullii. The maturation rate of reticulocytes. Blood. 1960; 15: 614–629.

[4] Johnstone R. M. et al. Reticulocyte maturation and exosome release: transferrin receptor containing exosomes shows multiple plasma membrane functions. Blood. 1989; 74: 5 1844–1851.

[5] Dertinger S. D. et al. Enumeration of micronucleated CD71-positive human reticulocytes with a single-laser flow cytometer. Mutation Research. 2002; 515: 3–14.

[6] Scientific Affairs, Sysmex Corporation. The Cell Analysis Center Scientific Bulletin Part 1 Cell analysis and bioimaging technology illustrated. 2007.

Sysmex Corporation
1-5-1, Wakinohama-Kaigandori, Chuo-ku, Kobe 651-0073, Japan · Phone +81 (78) 265-0500 · Fax +81 (78) 265-0524 · **www.sysmex.co.jp**

Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany · Phone +49 (40) 52726-0 · Fax +49 (40) 52726-100 · info@sysmex-europe.com · **www.sysmex-europe.com**