Cell Culture Sheet Used As a Chamber: Including Functional Test on Pig Monocytes

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Phagocytosis is usually observed on cover slips placed in culture, using leucocytes separated from whole blood. However, the method is costly, time-consuming and plagued. These factors place limitations on the application of these methods to the field of animal health. Therefore, simpler methods should be developed to measure phagocytic activity and other functions of leucocytes.

Here, I introduce a chamber termed cell culture sheet, and a simple test to measure the phagocytic and adhering activities of pig monocyte by the use of this chamber.

Materials and Methods

Description of cell culture sheets

The cell culture sheet consists of 3 parts; a glass or plastic plate, a sheet (adhesive tape), and a vinyl tube. The plate is a cleaned micro-slide glass, or an acrylic plastic plate for cell culture (26 × 76 mm). The sheet of tape which goes with this chamber, is adhesive tape (26 mm in width, Sekisui Co., Japan) which can bind on both sides. In the preparation of the culture sheet, a part of stick (a thin strip) which spreads a binder was masked with the tape removing the strip. Then, the masked tapes were cut (76 mm). Ten holes, each 5 mm in diameter were punched at 15 mm intervals. Transparent vinyl tubing from commercial sources was used. The tube is 2 mm thick with a 4 mm inner diameter (Fig. 1A). In general, the tubes were cut into lengths of 6 mm. Ten tubes were assembled on each sheet, after being glued to the surface of the slide glass. In short, the masked tapes were first removed from thin strips. Second, the tapes were strongly sealed on the slide glass. Finally, the vinyl tubes were combined with binder of sheet after removal of the reverse side of the seal.

Procedures of cell cultures

Pig blood (7 ml) was collected into a tube (12 by 100 mm) containing 100 units of...
of heparin. The tube was held in an upright position at room temperature for 20 min to allow the erythrocytes to settle, and leucocyte-rich plasma from 4 pigs was pooled in another tube. The cells, suspended in Hank's solution, were then added to each well. Each required approximately 20 µl and the inoculum should contain about 40,000 cells (about 3 µl of buffy coat). The culture sheets, kept within a slide box, were incubated at 37°C.

The leucocytes adhered to the slide glass within 30 min. Removal of culture medium and washing of cells were performed within the wells. The glutaraldehyde-treated sheep erythrocytes (20 µl), diluted 100 × with Hank's solution containing 10% calf serum, were poured into each well. After 30 min of further incubation, the cells

Fig. 1. Drawing showing construction of cell culture sheet (A), and photographs of cell culture sheet (B), showing the pig monocytes cultivated for 30 min by the use of the cell culture sheet (C), and phagocytizing or adhering to the fixed erythrocytes (D).
were washed in Hank’s solution in order to remove free erythrocytes. Then, the cells were fixed and stained in Giemsa solution. For microscopic observation, the sheet was lifted from the slide glass.

**Results and Discussion**

A culture sheet, assembled, in which leucocytes are cultured is shown in Fig. 1B. As already indicated by SOMMerville\(^3\), the microslide glass method is better for the manipulation of large numbers of samples for microscopic observation than the conventional cover slip method. Therefore, several types of chambers formed by attachment of a culture box or rings to a microslide and glass or plastic plate have been devised\(^4,5\) and manufactured\(^6\).

SOMMerville\(^3\) described a chamber assembled with a glass ring with mixtures of equal parts of paraffin wax and petroleum jelly. Ristic et al.\(^4\) also, described a chamber with plastic cups and a cement substance. My chamber was assembled using adhesive tape. The use of adhesive tape, as compared with the use of wax or cement, has several advantages. The assembly takes little time, if a punched adhesive tape is prepared, and 10 samples per slide can be tested at the same time using minute quantities (20 \(\mu l\)).

The microscopic picture of monocytes, cultured for 30 min in this sheet, is shown in Fig. 1C. Fig. 1D shows the monocytes phagocytizing or adhering to erythrocytes at 30 min after the addition of fixed erythrocytes. In the present study, the adhering cells consisted of monocytes (87.9%), neutrophils (7.3%), and others (4.7%). The phagocytic or adhering rate in monocytes was 28.1%. The conventional phagocytic test is performed using leucocytes separated from whole blood or organs. These procedures are time-consuming. The present test was performed by the use of the leucocytes in the buffy coat (about 3 \(\mu l\)) of whole blood. The procedure is very simple. Therefore, the method can be applied to detect disorders or impairment of phagocytic activity in pig monocytes.

However, this method cannot be applied to nonadhering phagocytic cells, such as most neutrophils, or the phagocytic cells of animals in which sedimentation of erythrocytes is extremely slow, such as in cattle or fowls.

The present test was performed during the first 60 min of incubation. Within that time, the sheet of the size described above should be applied for the detections of natural rosette cells, antibody-forming cells, FC receptor of monocytes or bacteria within leucocytes, etc. On the other hand, in a long term cell culture over 60 min, we recommend the use of the following culture sheet: 8 mm diameter of well, 7 mm inner diameter, 2 mm thick, 12 mm high tube and 50 \(\mu l\) medium volume per well. Such a sheet should be applied for detection of unknown viruses under suitable cell culture conditions.
References


