Ion Exchange Dish as a Tool for Cell Separation Based on Cell Surface Charge

Shoshi Inooka

Department of Animal Science, Faculty of Agriculture, Tohoku University, 1-1 Tsutsumidori Amamiya-Machi, Sendai 980, Japan

ABSTRACT. Methods of animal cell separation were studied based on cell surface charge. An ion exchange dish was devised by the author by coupling DEAE- or CM-Sephadex beads with polyacrylamide gel. Binding was shown of various animal cells to DEAE-Sephadex beads in the devised dish. The dissociation of bound mouse spleen lymphocytes was then examined using various reagents. More than 60% of adsorbed cells were dissociated by addition of a mixed solution of sodium citrate and Tween 80 with no detectable decrease in cell viability. The actual recovery of bound cells was also tested, and the step-flow method for elution was useful with solution containing sodium citrate, Tween 80, polybrene and EDTA at various concentrations of NaCl. The possibility of cell separation based on differences in cell surface charges was demonstrated in some cells.

Methods of cell separation based on such difference in physical properties as cell size, cell volume, cell density, adherent activity to solid substrates, e.g. antibody-bound non-ionic supports (1, 3, 8, 11, 12) and cell surface charge have been reported (2). Cell surface charges as suggested by the experiment on cell electrophoresis (9) may be useful in cell separation, though practical and convenient methods utilizing differences in cell surface charge have not been well established.

Ion exchangers containing side groups, e.g. diethylaminoethyl (DEAE) and carboxymethyl (CM), are naturally considered to be materials for separation based on the cell surface charge. However, useful methods utilizing this kind of material have not been developed. The ionic adsorption of cells to DEAE-Sephadex beads has been demonstrated (5, 6). However, a difficulty exists in detaching bound cells from the beads maintaining viability. The use of an ion exchange dish made by coupling ion exchange beads with polyacrylamide gel solves the problem. By the step-flow of the solution containing sodium citrate, Tween 80, polybrene and EDTA through the dish bound cells were dissociated from the beads without a decrease in cell viability. The results suggest that the ion exchange dish is useful for separating cells based on the difference in charges on the cell surface.

MATERIALS AND METHODS

Preparation of the ion exchange dish. The coupling procedures were: 2 ml of 60% acrylamide solution containing 0.4% N',N'-methylene-bisacrylamide, 0.1 ml of N,N,N',N'-tetramethylethylenediamine and 5 ml of 1% (NH₄)SO₂ were mixed and poured into a 90 mm

diameter plastic Petri dish. DEAE-(CM-) Sephadex beads (8 ml of a 0.25% suspension in water) were then layered over the gel solution before polymerization. After polymerization was completed, the remaining solution was decanted and the dish was washed with water twice to remove excess beads, then it was stored at 4°C until use.

Cell preparation. Erythrocytes were prepared from the fresh whole blood of sheep and fowl. Leucocytes of fowl were also prepared from the buffy coat of fresh whole blood with Alsvers' solution. Lymphocytes from the spleen of mouse and fowl, and from the bursa of fabricius and the thymus of chick were prepared by mincing each organ. Macrophages were prepared from the peritoneal fluid of DD mice by washing with saline. Ehrlich tumor cells were maintained in the form of ascites with DD mice. BHK cells, PS cells and L cells grown in Eagles MEM supplemented with 10% serum were dispersed in 2% EDTA. These cells were washed twice with phosphate buffered saline, then washed with 0.15 M NaCl in 0.01 M sodium phosphate, pH 7.1. In the cell dissociation experiments the lymphocytes of mouse spleen were used. Also, the lymphocytes of mouse and fowl, the macrophages of mouse and the erythrocytes of fowl were used in the cell elution experiments.

Cell binding. An 8 ml suspension of each cell type (containing 8×10^6 total cells in 0.15 M NaCl) was pipetted gently onto the dishes and allowed to stand at 20°C for 90 sec. The aqueous layer containing unattached cells was decanted and the dish was washed twice with 0.15 M NaCl to remove loose cells. Bound cells were observed under a microscope and the total cell number bound per bead was counted. The degree of binding are: $\#\sim\#$ over 50 cells; $+$ between 50–10 cells; $-$ below 10 cells per bead.

Materials tested for cell dissociation and elution. Materials were tested on their ability to dissociate cells from the ion exchange dish. Sodium chloride, ammonium sulfate, acetic acid, glycine, sodium citrate, lithium sulfate, sodium sulfate, dioctyl sodium-sulfosuccinate, sodium deoxycholate, sodium lauryl sulfate, Triton X-100, Tween 80, benzethonium chloride, heparin, polybrene, protamine sulfate and dextran sulfate were tested. Mixtures of trypsin and Triton X-100, Tween 80 and trypsin, trypsin and EDTA, sodium citrate and Triton X-100, sodium citrate and Tween 80, sodium citrate and EDTA, heparin and Triton X-100, heparin and Tween 80, heparin and EDTA were also examined.

In the cell elution experiments, the solution of sodium citrate, Tween 80, EDTA and polybrene in 0.10 (osmotic pressure modulated by sucrose), 0.15, 0.20, 0.25, 0.30 and 1.50 M NaCl (pH 6.7) were used.

Cell dissociation. To test the effect of the various reagents on cell dissociation lymphocytes prepared from mouse spleen were washed twice with phosphate buffered saline. To test the effect of a single reagent, cells were bound to the DEAE-Sephadex beads in a 0.15 M NaCl solution as described above. Recovery was made with 0.15 M NaCl containing the reagent to be tested. To test the effect of coupled reagents, cells were bound to beads in a 0.05 M NaCl solution (osmotic pressure modulated by sucrose) and dissociation was tested in 0.30 M NaCl solution. After unbound cells had been removed, the solution containing the reagents was pipetted gently onto the dishes. The whole was allowed to stand at 20°C for 5 minutes; then after vigorously shaking the dish, the number of loose cells was counted under a microscope. Results of cell dissociation were calculated as follows:

$$\frac{\text{Number of dissociated cells due to treatment with reagents}}{\text{Number of bound cells before treatment with reagents}} \times 100$$

Rates of dissociation are: $\#$ (over 60%), $\#$ (between 60 and 40%), $+$ (between 40 and 30%), and $-$ (under 30%).

Elution of bound cells. The DEAE-Sephadex ion exchange dishes were twice washed with

0.05 M NaCl as described above. The washed cells were bound to the DEAE-Sephadex beads by an addition of 8 ml of the cell suspension (10^6 – 10^7 cells/ml of 0.05 M NaCl) after which the dishes were kept at 20°C for 90 sec. Elution of bound cells after the removal of unbound cells was carried out by the step-flow of 8 ml of a solution composed of sodium citrate (2%), Tween 80 (0.001%), EDTA (2.0%) and polybrene (0.01%) in solutions each containing 0.10, 0.15, 0.20, 0.25, 0.30 and 1.50 M NaCl. Each flow was carried out at 20°C for 5 minutes, and each eluate was collected. The total number of cells eluted was counted with a hemacytometer and cell viability was judged from the results of trypan blue elution tests.

RESULTS

Cell binding. The adsorption ability of the ion exchange dishes was tested to determine whether the DEAE- or CM- ion exchange group prepared using polyacrylamide gel as the coupling material could best adsorb cells. Results are summarized in Table 1. The data show that all the cells tested bound to the dish with DEAE-Sephadex beads, but not to the dish of CM-Sephadex beads and the polyacrylamide gel layer except for erythrocytes. As shown in a previous paper (5), the binding of cells reached a maximum within 90 sec. There appears to be little difference in behavior and in the number of cells between the exchanger dish and the tube which has been previously described (5). This observation shows that coupling is carried out with no formation of a new ion exchange group and with no destruction of the function of the original ion exchange group of beads.

Effect of several reagents on cell dissociation. To dissociate bound cells an experiment was performed using several reagents which were expected to weaken the bonds between the cells and the ion exchange group in the dish. Table 2 shows that the systems of sodium chloride (18%), sodium citrate (2 or 20%), lithium sulfate (0.1%), Tween 80 (0.001%) and heparin (5%), dissociated about 30–40% of the adsorbed cells. With dioctylsodium sulfosuccinate, sodium lauryl sulfate, Tween 80 (0.01%) and benzethonium chloride, even higher rates of dissociation occurred. The

TABLE 1. ADSORPTION OF SOME KINDS OF ANIMAL CELLS TO DEAE-SEPHADEX BEADS IN ION EXCHANGE DISHES

Cells tested	DEAE-Sephadex	CM-Sephadex
Sheep erythrocytes	+++	+
Fowl erythrocytes	+++	+
Fowl leucocytes	++	—
Fowl spleen lymphocytes	++	—
Mouse spleen lymphocytes	++	—
Ehrlich ascites cells	+++	—
Mouse macrophages	+++	—
BHK cells	++	—
PS cells	++	—
L cells	++	—

Washed animal cells were suspended in 0.15 M NaCl solution, pH. 7.1. Cell suspensions were poured into dishes which had been coupled with DEAE- or CM-Sephadex beads. The samples were kept for 90 sec at 20°C, then after washing off unadsorbed cells the number of cells adsorbed was counted under a microscope. The degree of cell binding is: +++~++ (over 50 cells), + (between 10 and 50 cells), and — (below 10 cells) per bead.

TABLE 2. DISSOCIATION CELLS BOUND TO THE ION EXCHANGE DISHES BY SOME SALT SOLUTIONS, DETERGENTS, POLYCATIONS, AND POLYANIONS

Salt solution			Detergents			Polycations and polyanions		
Reagents tested	Concentration (%)	Dissociation	Reagents tested	Concentration (%)	Dissociation	Reagents tested	Concentration (%)	Dissociation
Sodium chloride	1.8	—	Dioctyl sodium sulfo-succinate	0.01	—	Heparin	5.0	+
	18.0	+		0.1	++		10.0	—
Ammonium sulfate	0.1	—	Sodium deoxycholate	0.01	—	Polybrene	50.0	+
	2.5	—		0.1	—		0.01	—
Acetic acid	0.1	—	Sodium lauryl sulfate	0.01	—	Protamine	0.1	—
	1.0	—		0.1	++		0.01	—
Glycine	0.1	—	Triton X-100	0.001	—	DEAE-dextrane	0.01	—
	1.0	—		0.01	—		0.1	—
Sodium citrate	2.0	+	Tween 80	0.001	++			
	21.0	+		0.01	++			
Lithium sulfate	0.1	+	Benzethonium chloride	0.01	—			
	1.0	—		0.1	++			
Sodium sulfate	0.1	—						
	1.0	—						

Washed lymphocytes prepared from mouse spleen were adsorbed on DEAE-Sephadex beads in a Petri dish in 0.15 M NaCl solution. Eight ml of a solution containing the reagents indicated in the table was then poured into the dish. After 5 minutes cell dissociation was assayed under a microscope. The degree of cell dissociation in Tables 2 and 3 is: ++ (over 60%), + (between 40 and 60%), — (under 30%).

TABLE 3. DISSOCIATION OF BOUND CELLS BY COUPLED REAGENTS

Solution Mixture		Dissociation
Trypsin (0.2%)	Triton X-100 (0.001%)	+++
	Tween 80 (0.001%)	++
	EDTA (2.0%)	++
Sodium citrate (2%)	Triton X-100 (0.001%)	++
	Tween 80 (0.001%)	+++
	EDTA (2.0%)	++
Heparin (5%)	Triton X-100 (0.001%)	+
	Tween 80 (0.001%)	--
	EDTA (2.0%)	--

Washed lymphocytes prepared from mouse spleen were adsorbed on the dish in 0.05 M NaCl solution. Eight ml of a mixture of the reagents in 0.30 M NaCl solution was poured into the dish. After 5 minutes cell dissociation was assayed under a microscope.

Fig. 1. Elution of bound cells by the step flow of a solution. Washed cells were bound to DEAE-Sephadex beads in 0.05 M NaCl solution. Eight ml of the solution mixture of Tween 80 (0.001%), sodium citrate (2%), EDTA (2%) and polybrene (0.01%) in 0.10, 0.15, 0.20, 0.25, 0.30 and 1.50 M NaCl solution, pH 6.7, was poured into the dish, at 5 minute intervals. The total number of cells in each eluate was counted. a) mouse spleen lymphocytes (open column) and fowl erythrocytes (solid column) b) mouse macrophages.

Fig. 2. Elution of bound lymphocytes of bursa and thymus by the step-flow of a solution. Washed lymphocytes of the bursa and the thymus were bound to DEAE-Sephadex beads in 0.05 M NaCl solution. Eight ml of the elution was poured into the dish at 5 minutes intervals. Fractions of the same type were pooled and the total number of cells was counted. Open column, Thymus cells; Solid column, Bursa cells.

latter results may be due to the destruction of adsorbed cells because these reagents were cytotoxic at the concentration tested.

Effects of coupled reagents on cell dissociation. To obtain a higher yield than that for dissociation with single reagents, the effects of coupled reagents were tested. Results are given in Table 3. In the mixture systems of trypsin and Triton X-100, and of sodium citrate and Tween 80, more than 60% of the bound cells were dissociated from the beads. In contrast, incubation with the other coupled reagents resulted in cell dissociation of less than 60%.

Elution of bound cells. Since results of the dissociation of lymphocytes from the ion exchange dish with the coupled reagents were good, the elution of bound lymphocytes, erythrocytes and macrophages from the dishes was tested using a flow of NaCl solution at various concentration containing polybrene, EDTA, sodium citrate and Tween 80. Polybrene was added to prevent readsorption on the DEAE-Sephadex beads once cells were loosened. EDTA was added to promote the loosening of cells. Results are shown in Fig. 1. Mouse spleen lymphocytes and fowl erythrocytes elutions were dependent on the concentration of NaCl. The recovery of mouse spleen lymphocytes (Fig. 1a) shows a peak at 0.30 M NaCl as does that of fowl erythrocytes (Fig. 1a). Macrophages (Fig. 1b) were eluted mainly with 0.15 M and 1.5 M NaCl solutions. Viability of the eluted cells in Fig. 1a–1c was more than 90% in all the fractions collected, based on results of the trypan blue elution test. Total cell recovery calculated from the eluted cells was about 70% of the total bound cells (Fig. 3).

To test the possibility of cell separation, elution patterns for the lymphocytes of the bursa and the thymus were determined. Lymphocytes of the bursa were eluted mostly in flowing NaCl solutions with concentrations lower than 0.25 M. In contrast, thymus cells were eluted mainly in flowing NaCl solutions with concentrations higher than 0.25 M (Fig. 2).

DISCUSSION

The usefulness of the ion exchange dish as a tool for the separation and manipula-

Fig. 3. Photo micrographs of the surface of the ion exchange dishes in the step-flow of a solution. Large particles are DEAE-sephadex beads and small particles are cells bound to the beads. a) dish with adsorbed mouse macrophages in 0.05 M NaCl. b) dish with adsorbed mouse macrophages in 0.05 M NaCl, which had then been washed with 1.5 M NaCl. c) dish with adsorbed fowl erythrocytes in 0.05 M NaCl. d) dish with adsorbed erythrocytes in 0.05 M NaCl, which had then been washed with 1.5 M NaCl. $\times 100$

tion of cells, based on cell surface charges and methods of cell recovery from the support, was demonstrated.

The use of beads on Petri dishes has several advantages over columns:

- 1) The time for adsorption and for the removal of cells is less.
- 2) Sterile handling is possible.
- 3) The attachment to and the removal of the cells from the beads can be directly observed.

These are obviously valuable for cell yields and maintenance.

Several materials such as agarose, collagen and gelatin can be used in procedures for coupling the beads with the dishes. Polyacrylamide gel was used in the present experiments as a coupling reagent to prepare the ion exchange dishes. Characteristics of the ion exchange dishes were studied using various cells. The binding of cells to DEAE-Sephadex beads occurs within 90 sec at 20°C , and within the time the binding

of cells to polyacrylamide gel or CM-Sephadex beads do not occurs. The observed adsorption was similar to results described previously (6). This suggests that the ion exchange support is useful for cell-handling. However, it is desirable to examine whether the use of DEAE-Sephadex in various kinds of ion exchangers and whether the use of polyacrylamide gel in various coupling materials are best.

Previous attempts to recover cells by elution from the resin with a salt solution produced very poor results, because a high concentration of salt was required. Theoretically it may be possible to separate cells from the support as follows:

- 1) by destroying the support itself or the materials coating the support
- 2) by a disappearance of the charges or materials on the cell surface
- 3) by varying or substituting the binding charge between the DEAE-ion exchange group and the cells

Scholssman et al. (10) tried dextranase in the separation of immunocytes from the antigen coat Sepharose. They reported a high yield of cells obtained over a long treatment period with the enzyme. Kedar (7) also was successful in separating immunocytes from an erythrocyte coated support by lysis of the erythrocytes. But recovery of the cells by digesting the substances on the cell surface was not very successful because cell recovery was not observed for the majority of cells when bound cells were treated in solutions of trypsin, collagenase and other enzymes. I believe the binding between the ion exchange groups of the resin and the cell surface is very strong and complex. However, I observed that Ehrlich cells bound with DEAE-Sephadex beads were easily recovered with trypsin (unpublished data). This phenomenon has not yet been investigated, but it suggests that special cells may be recovered by using special enzymes or reagents. It was also difficult to recover cells by substituting the charge of the binding with salts as described above. A test of various reagents demonstrated that a solution of a mixture of sodium citrate and Tween 80 was best for dissociating cells without a decrease in cell viability. In the elution experiments with lymphocytes of brusa and thymus, however, a little difference in the NaCl concentration was needed for elution (Fig. 2). The elution patterns from the ion exchange dishes may have some correlation with the actual net charge of the lymphocytes of the brusa and thymus cells, because the elution behavior of both paralleled the electrophoretic mobility of each cell (4). The ion exchange dish should be a useful method for cell separation and manipulation. Application methods are being investigated.

Acknowledgments. Thanks are due to Dr. M. Yoshida for his discussion and reading of the manuscript, and to the Eiken Kizai Co., Tokyo, for the preparation of the ion exchange dishes.

REFERENCES

1. CHIPOWSKY, S., Y.C. LEE and S. ROSEMAN. Adhesion of cultured fibroblasts to insoluble analogues of cell surface carbohydrates. *Proc. Nat. Acad. Sci. USA* **70**, 2309-2312, 1973
2. CUTTS, J.H. Cell separation methods in hematology. Academic press, New York, 1970
3. EDELMAN, G.M., U. RUTISHAUSER and C.F. MILLETTE. Cell fractionation and arrangement on fibers, beads, and surfaces. *Proc. Nat. Acad. Sci. USA* **68**, 2153-2157, 1971
4. HANJAN, S.N.S. and G.P. TALWAR. The selective action of poly AU on the electrophoretic mobility and surface charge of a subpopulation of B cells. *J. Immunol.* **114**, 55-58, 1975
5. INOOKA, S. The adsorption of suspended MH129F cells to DEAE-Sephadex particles. *Tohoku. J. Agr. Res.* **20**, 19-26, 1969
6. INOOKA, S. The adsorption of some kinds of cells to DEAE-Sephadex particles. *Tohoku. J. Agr. Res.* **20**, 128-131, 1969

7. KEDAR, E., M. ORTIZ de LANDAZURI and B. BONAVIDA. Cellular immunoadsorbents: A simplified technique for separation of lymphoid cell population. *J. Immunol.* **112**, 1231–1243, 1974
8. OKA, T. and Y.J. TOPPER. Insulin-sepharose and the dynamics of insulin action. *Proc. Nat. Acad. Sci. USA* **68**, 2066–2068, 1971
9. PURBOM, M.L., E.J. AMBROSE and G. KLEIN. A correlation between electrical surface charge and some biological characteristics during the stepwise progression of a mouse sarcoma. *Nature* **181**, 1586–1587, 1958
10. SCHOLSSMAN, S.F. and L. HUDSON. Specific purification of lymphocyte populations on a digestible immunoadsorbent. *J. Immunol.* **110**, 313–315, 1973
11. TRUFFA-BACHI, P. and L. WOFSY. Specific separation of cells on affinity columns. *Proc. Nat. Acad. Sci. USA* **66**, 685–692, 1970
12. WIGZELL, H. and B. ANDERSSON. Cell separation on antigen-coated columns. Elimination of high rate antibody-forming cells and immunological memory cells. *J. Exp. Med.* **129**, 23–36, 1969

(Received for publication, September 26, 1978)