Biological Separations

Methods of Cell Separation

Volume 1

Edited by Nicholas Catsimpoolas

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Preface

Presently, the need for methods involving separation, identification, and characterization of different kinds of cells is amply realized among immunologists, hematologists, cell biologists, clinical pathologists, and cancer researchers. Unless cells exhibiting different functions and stages of differentiation are separated from one another, it will be exceedingly difficult to study some of the molecular mechanisms involved in cell recognition, specialization, interactions, cytotoxicity, and transformation. Clinical diagnosis of diseased states and use of isolated cells for therapeutic (e.g., immunotherapy) or survival (e.g., transfusion) purposes are some of the pressing areas where immediate practical benefits can be obtained by applying cell separation techniques. However, the development of such useful methods is still in its infancy. A number of good techniques exist based either on the physical or biological properties of the cells, and these have produced some valuable results. Still others are to be discovered. Therefore, the purpose of this open-end treatise is to acquaint the reader with some of the basic principles, instrumentation, and procedures presently in practice at various laboratories around the world and to present some typical applications of each technique to particular biological problems. To this end, I was fortunate to obtain the contribution of certain leading scientists in the field of cell separation, people who in their pioneering work have struggled with the particular problems involved in separating living cells and in some way have won. It is hoped that new workers with fresh ideas will join us in the near future to achieve further and much needed progress in this important area of biological research.

Nicholas Catsimpoolas

Cambridge, Massachusetts

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1

Preparative Density Gradient Electrophoresis and Velocity Sedimentation at Unit Gravity of Mammalian Cells

NICHOLAS CATSIMPOOLAS AND ANN L. GRIFFITH

I. DENSITY GRADIENT ELECTROPHORESIS

A. Introduction

In 1975 we described a new method for the preparative separation of mammalian cells by density gradient electrophoresis (Catsimpoolas and Griffith, 1975; Griffith *et al.*, 1975). This development came about by combining the use of an isoosmolar Ficoll–sucrose density gradient medium (Boltz *et al.*, 1973, 1976) with a commercially available apparatus originally designed for polyacrylamide gels (Jovin *et al.*, 1964) and a fraction collection method involving differential pumping velocity of the chase and density gradient fluids (Svendsen, 1972). The technique can be used to separate highly viable and functional mammalian cells—in bulk quantities, i.e., up to 10^8 cells—if they exhibit different surface charge and therefore electrophoretic mobility. This capability has been demonstrated in several recent reports from this laboratory (Catsimpoolas *et al.*, 1976a; Ault *et al.*, 1976; Platsoucas *et al.*, 1976; Griffith *et al.*, 1976).

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B. Apparatus and Procedures

1. The Electrophoresis Apparatus

The Buchler Poly-Prep 200 apparatus (Buchler Instruments, Fort Lee, N.J.) is used for the density gradient electrophoresis experiments. A schematic diagram of the Poly-Prep 200 is shown in Fig. 1. The apparatus has a glass separation chamber with an outer cooling jacket and an inner cooling glass piece that is inserted into the center of the column. An annulus (hollow cylinder) is thus produced having a cross-sectional area of 17.6 cm^2. The gradient formation and subsequent cell fractionation takes place within this annulus. A rigid porous glass membrane separates the lower buffer reservoir (positive electrode) from the separation chamber. Inlet ports are available at the bottom of the separation column and a narrow-bore outlet is provided in the center of the inner cooling piece. Thus, the bottom of the gradient can be pumped upward through this narrow channel and into the fraction collector tubes.

2. Washing the Column

All glass surfaces that are in contact with the cells are washed thoroughly by immersion in 7× (Linbro Chemical Co., New Haven, Conn.) and rinsed several times with hot tap water, followed by three distilled water rinses and air drying. The annulus is subsequently siliconized with a 1% solution of Siliclad (Clay Adams, Parsippany, N.J.) in water. The column is filled with the siliconizing solution, drained slowly (20 min), rinsed extensively with distilled water, and air dried. Sterilization of the electrophoresis chamber can be carried out by rinsing with a solution containing 1% formaldehyde, 0.2% chlordioxide followed by several rinsings (3 to 5 liters) of sterile water (Wigzell and Häyry, 1974).

3. Preparation of Solutions

Stabilization of cells during electrophoresis is achieved by a 2.5–6.25% Ficoll (400,000 MW, Pharmacia Fine Chemicals) gradient (Fig. 2), which is also an inverse 6.35–5.72% sucrose gradient (Boltz *et al.*, 1973) covering the density range 1.0397–1.0480 g/cm^3 at 4°C and is isoosmolar (300 mOsmol) throughout. Prior to the formation of the gradient 25 g Ficoll are dissolved in 200 ml of the *electrophoresis buffer* and dialyzed against 5000 ml of the same buffer overnight at 4°C. Ficoll is dissolved by magnetic stirring with intermittent application of vacuum at approximately 20 mm Hg for 10 min. The dialyzate is then made up to 250 ml (10% Ficoll solution)

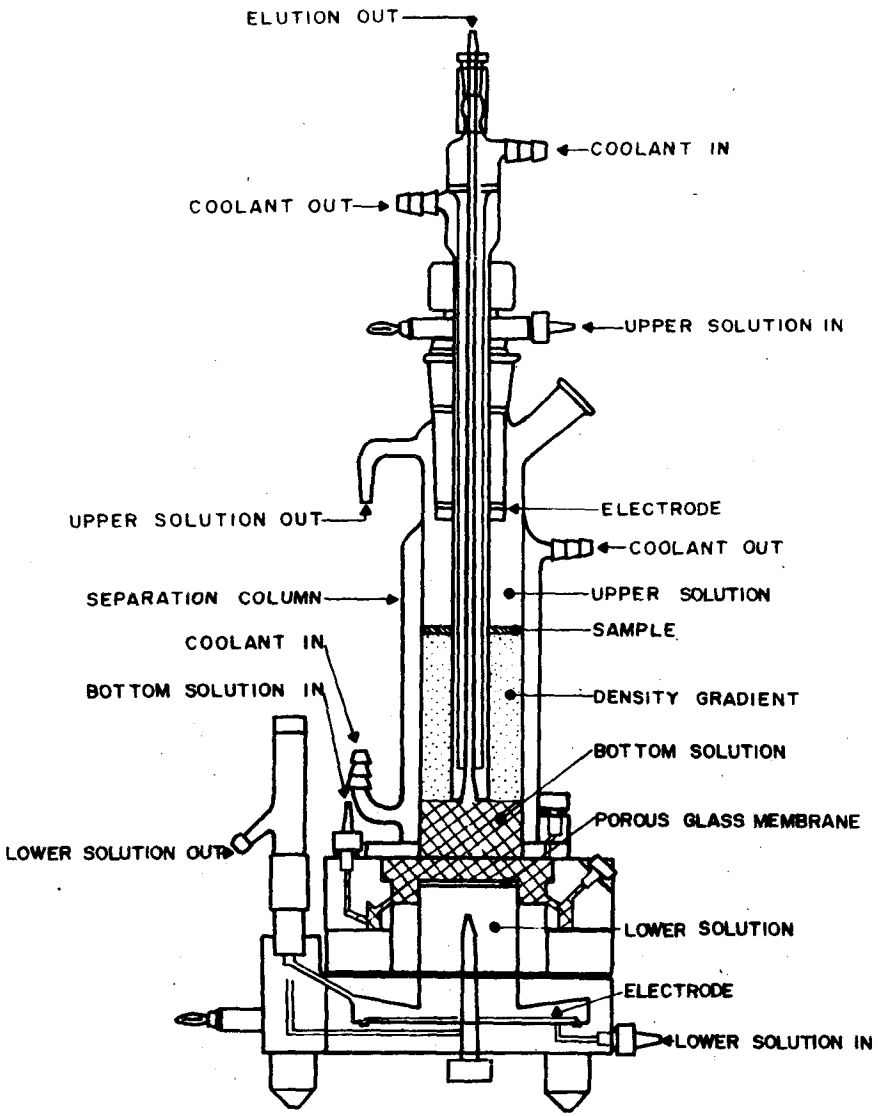

FIGURE 1. Schematic diagram of the preparative electrophoresis column (Buchler Poly-Prep 200).

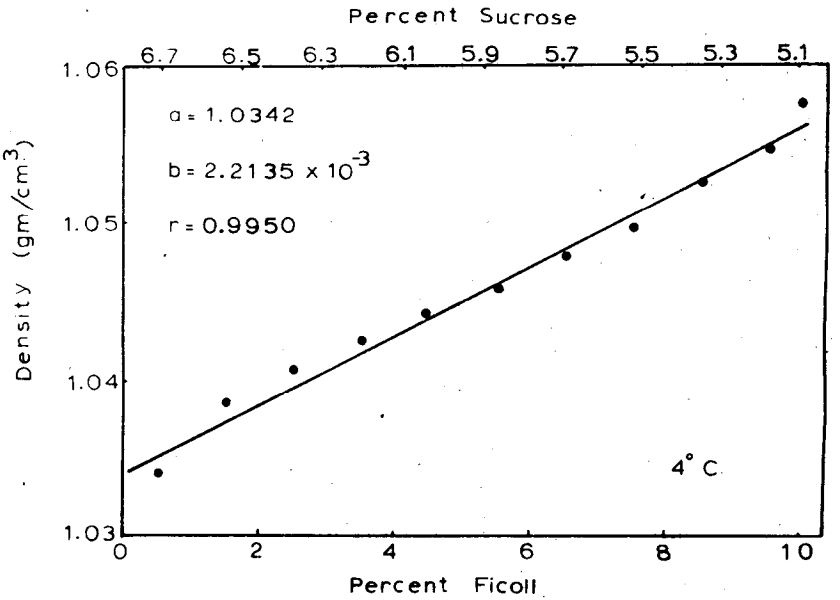

FIGURE 2. Density of the Ficoll–sucrose gradient at 4°C.

with freshly prepared *electrophoresis buffer*. Dialyzing the Ficoll dramatically reduced cell clumping. The light solution (2.5% Ficoll, 6.35% sucrose) is prepared as follows. Sucrose (3.175 g) is added to 12.5 ml of the stock 10% Ficoll solution and made up to 50 ml with the *electrophoresis buffer*. Similarly, the *dense solution* (6.25% Ficoll, 5.72% sucrose) is prepared by the addition of 2.862 g of sucrose to 31.25 ml of the stock 10% Ficoll solution and made up to 50 ml with the buffer. The *sample solution* (2.0% Ficoll, 6.44% sucrose) is made by dissolving 0.644 g sucrose in 2 ml of the 10% Ficoll solution and made up to 10 ml with the buffer. The *bottom solution* (10% Ficoll, 5.1% sucrose) is prepared by dissolving 10.2 g sucrose in 150 ml of the 10% Ficoll solution and made up to 200 ml with the same stock solution. The *upper electrode solution* (6.8% sucrose) is prepared by dissolving 136 g sucrose in 1000 ml of the *electrophoresis buffer* and made to 2000 ml with the same buffer. The *lower electrode solution* (5.1% sucrose) consists of 76.5 g sucrose made up to 1500 ml with the *electrophoresis buffer*.

The *electrophoresis buffer* adjusted to (pH 7.4) has the following composition: 0.20 g KCl, 1.5 g Na_2HPO_4, 0.20 g KH_2PO_4, 0.12 g sodium acetate, 10.0 g glucose made to 1000 ml with glass distilled water. Usually,

10 liters of the buffer are prepared for each experiment. This buffer is a modification of that described by Boltz *et al.* (1973). The specific conductivity of the buffer should be 1.026 mmho/cm at 4°C at an ionic strength of 0.023 M.

4. *Experimental Procedure*

The overall arrangement of solutions in the Poly-Prep 200 column is shown in Fig. 3. The experimental procedure for density gradient electrophoresis is as follows. Part B (1) of the lower buffer/electrode section (2) is filled via the lower solution inlet port (3) with deaerated *lower electrode solution* (Fig. 4). The air bubble that forms directly below the porous glass membrane is removed by tilting this part so that it escapes into the lower reservoir area. Part A (4), containing a circular channel communicating with six equidistantly spaced ports, is screwed onto part B. The entire section is then leveled by means of two adjustable screws located on part B and the spirit level found on part A. The column assembly (7) is placed on top of part A, being careful to align the red dots, and securely fastened with four sliding adjustable clamps located on part A. The internal glass cooling tube (12) containing the central elution capillary (13) is inserted into the column assembly and adjusted so that its lower surface is approximately 2.5 cm above the porous glass membrane. The coolant out-port (8) of the assembly and the coolant in-port (15) of the internal glass cooling tube are interconnected. A cooler adjusted to 4°C is connected to the coolant in-port (9) of the column assembly and to the coolant out-port (14) of the internal glass cooling tube, and circulation is begun. Deaerated *bottom solution* (~80 ml) is introduced into the column assembly to the level of the lower surface of the internal glass cooling piece through the bottom solution inport (5), filling the circular channel and entering the column assembly interior through the six ports of part A of the lower buffer electrode section.

A multiple entry port is used to block the side tube (11) of the column assembly. A tubing assembly consisting of 45 cm of 1.0 mm i.d. Teflon tubing, 10 cm of 2 mm i.d. Tygon tubing, and 2 cm of 4 mm i.d. Tygon tubing is inserted through a small hole in this port so that only the Teflon tubing is located in the column interior. This tubing assembly is used to layer the *density gradient*, *upper electrode solution*, and *sample*, in that order. The tubing assembly is filled with *bottom solution*, clamped, and attached to a gradient maker (LKB 8121, LKB Produkter AB, Bromma, Sweden) containing 50 ml of the *dense solution* and 50 ml of the *light solution*. The *density gradient* (100 ml) is formed in the column assembly at

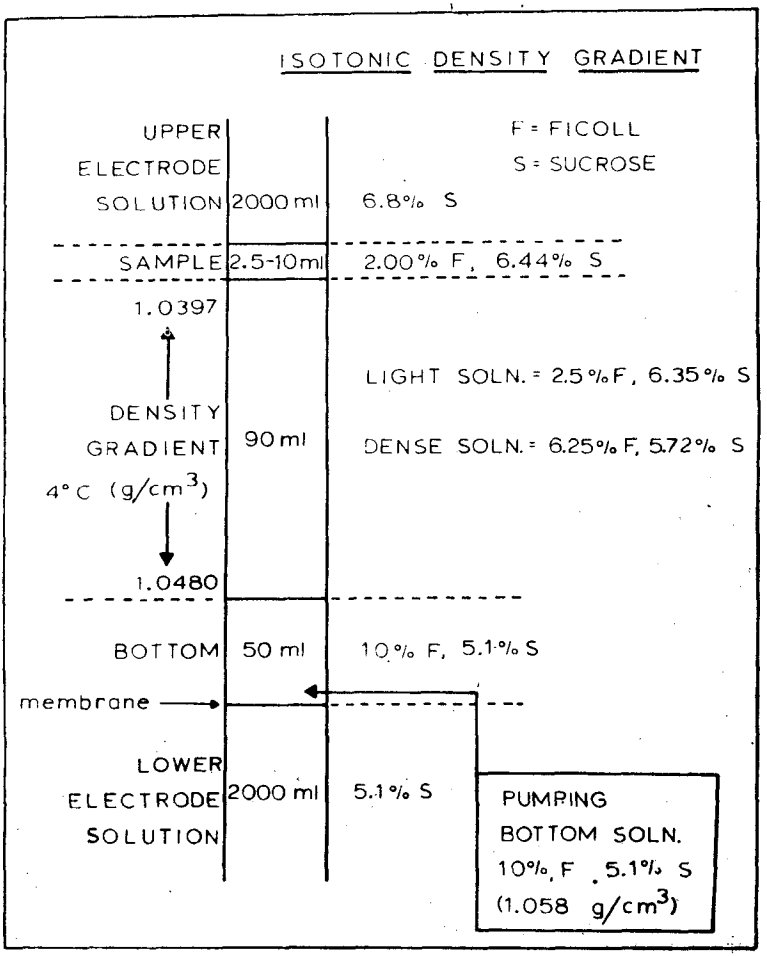

FIGURE 3. Schematic diagram of the arrangement of solutions in the preparative electrophoresis column.

a rate of 3 ml/min. The tubing assembly is clamped just before it empties and is attached to a reservoir (e.g., syringe body) containing *upper electrode solution*. The *upper electrode solution* (~125 ml) is layered with increasing speed on top of the gradient until the upper electrode is covered. Again the tubing assembly is clamped just before emptying and attached to a cooled 10 ml syringe, containing the *sample solution* (5–10 ml). The *sample solution*, containing 1–10 × 10^6 cells/ml, usually 5 × 10^6/ml, is