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Optimal use of the cationic polyelectrolyte poly-L-lysine in the preparation of cell monolayers for diagnostic cytopathology

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The membrane surface of most, if not all, cells bears a net negative charge owing to the presence of anionic radicals covalently linked to the molecular matrix of the cell membrane. Studies of these anionic sites and their interaction with polyelectrolytes led to the discovery of useful ionic properties—namely, cell to cell adhesion and aggregation.^{1–7}

This adhesive property of ionic interaction between the cell membrane and a cationic polyelectrolyte has been applied to the preparation of biological materials for in vitro studies of bioelectric responses and contact inhibition, electron microscopy, diagnostic cytopathology, and automated screening for cervical cancer.^{8–13}

The problems of variable thickness of cell smears and cell overlap for automated scanning have been resolved by laying a monolayer of cells on to a glass slide precoated with the cationic polymer poly-L-lysine hydrobromide. But this technique has not met with universal acclaim, which may well be due to the use of the poly-L-lysine at suboptimal conditions for molecular weight, concentration, temperature, and pH. We have attempted to determine these optimal conditions.

Material and methods

We investigated three different molecular weights of poly-L-lysine hydrobromide—that is, 100 000, 220 000, and 500 000 (Sigma London Chemical Co). The effects of variation in pH and temperature at different concentrations of polymer were studied.

PREPARATION OF SLIDE SURFACES

Each polymer was prepared at concentrations of 0.01, 0.1, and 1.0 g/100 ml at pH values of 4, 6, 7, 8,

10, and 12. The polymers were then spread evenly over glass slides with a Pasteur pipette and left to dry before use. (Figs. 1a and b).

PREPARATION OF CERVICAL SAMPLES AND MONOLAYERS

Cervical suspension samples were taken into 1% acetic acid, 10% alcohol in Hank's balanced salt solution. Cell aggregates were dispersed using the syringe technique of Husain *et al.*¹³ In order to provide sufficient material for a comparative trial 20 cervical samples were pooled after the syringing

Fig. 1 (a, b) Aqueous solution of polymer is spread evenly over the slide with a Pasteur pipette.

(c) An area of 2.5 cm² is demarcated in the centre of the slide using a black felt pen.

(d) A 250 µl aliquot of cell suspension dosed and settling on to the slide.

process. The pooled cells were divided into six aliquots and each aliquot of cells was washed in buffer using the same pH range as for the preparation of the polymers. A suspension of buffer and packed cells (5:1) was made at each pH value and 250 μ l samples were dosed on to corresponding coated slides. Each dose covered an area of 2.5 cm², which had been demarcated with a black felt pen (Fig. 1c). The cells were allowed to settle for 5 min at 1g, covered over with a petri dish to avoid evaporation and contamination (Fig. 1d). Excess cells were then washed off in buffer at the appropriate pH value; slides were fixed in carbowax for 5 min and then left to dry.

A similar experiment was conducted on slide surfaces prepared as before except that poly-L-lysine solutions were prepared in distilled water at pH 7. Cell suspensions were dosed again in phosphate buffered saline at pH 7.4. To assess the effect of temperature variation slides, dosed and settled at 37°C on a hot plate, were compared with those treated at 20°C on the bench.

STAINING

Slides in both experiments above were stained for 5 min using a rapid gallocyanin chromic potassium sulphate technique.¹⁴

ASSESSMENT OF CELL ADHERENCE

The total number and distribution of cells adhering to each slide was assessed using the CERVIFIP automated cervical cytology prescreener.¹⁵

Results

In the studies on the effect of pH optimal cell adherence was observed at pH7 for poly-L-lysine at

Fig. 2 Mean number of cells/mm² adhering to slides coated with varying molecular weights of poly-L-lysine at different values of pH.

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Fig. 3 Mean number of cells/mm² adhering to slides coated with poly-L-lysine (MW 500 000) at concentrations of 0.01, 0.1, and 1.0 g/100 ml at 37°C and 20°C.

500 000 MW (Fig. 2). In temperature variation studies, adherence was best on slides coated with poly-L-lysine 500 000 MW prepared using distilled water at a concentration of 0.1 g/100 ml, dosed and settled for 5 min at 37°C (Fig. 3).

Discussion

These experiments with cervical material show that poly-L-lysine is useful for the preparation of cell monolayers. We have also applied this technique to other cytological cell suspension samples—for example, breast cyst fluid, urine, and serous effusions—and have achieved similar results. With this method the cells are settled under a relatively mild force of 1g, which eliminates the possibility of artefact due to compression sometimes seen in membrane filtration or centrifugal systems and permits the rather weak negative charge on cells to act also in mutual repulsion. In fact, monolayers can be achieved which minimise cell overlap, thus enhancing the morphological appearance of the cells, which is of value in both diagnostic and automated cytopathology.

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Semiautomated method for quantitative urine culture

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Examination of urine must be the most frequently performed task in any bacteriology laboratory with a large service commitment. Generally, an attempt is made to determine the numbers of viable bacteria present in the specimen, and a variety of methods have been used to this end. At the time of publication of his papers in the 1950s,¹⁻³ from which the criteria for interpretation of bacterial counts in urine samples were defined, Kass used the agar pour plate method. Hoeprich, in 1960,⁴ introduced the use of the calibrated loop for making a surface count of organisms on agar media (this method had originally been described in 1928⁵ as a means of enumerating bacteria in samples of milk). In 1964⁶ Leigh and Williams introduced the blotting paper strip method. Finally, in 1965⁷ Mackey and Sandys took the business of inoculation outside the laboratory when they published their paper outlining the use of dipspoons.

All of these methods remain in use. Some modifications have been made, most especially in the method of Mackey and Sandys with the introduction of commercial "kits," some bearing two types of indicator medium and others using material other than agar as a support.⁸ We do not wish to discuss

the merits or demerits of the breakpoints commonly used in counting bacteria in urine. Discussions concerning such breakpoints, however, are valid only when they are founded on results which do not vary from operator to operator (and from laboratory to laboratory). In this paper we present a method which we believe approaches this ideal more closely than others currently in use.

Material and methods

A Hamilton MicroLab M programmable diluter (VA Howe and Co, London) (Fig. 1) was programmed to perform the sequence of functions shown in the Table. Urine specimens are mixed by inversion. Seventy microlitres is withdrawn and 60 μ l is dispensed into one well of a microtitre tray for estimation of the number of cells using an inverted microscope.⁹ The remaining 10 μ l of urine is dispensed with 990 μ l of diluent (sterile peptone) into a 1.5 ml autoanalyser cup (Sarstedt Limited, Leicester); the vortex produced achieves mixing automatically. Then 120 μ l of the diluted sample is taken up and dispensed as six 20 μ l drops on to a cysteine lactose electrolyte deficient (CLED) agar plate in the pattern shown in Fig. 2. The sixth drop is spread using the tip of the dispenser. All the manipulations above are performed using the automatic diluter.

Program for the Hamilton Microlab M

- 1 Automatically pick up 1000 μ l peptone
- 2 Automatically deliver 1000 μ l peptone
- 3 Manually pick up 70 μ l specimen
- 4 Manually deliver 60 μ l specimen to microtitre tray
- 5 Automatically pick up 990 μ l peptone
- 6 Manually deliver 1000 μ l diluted specimen
- 7 Manually pick up 120 μ l diluted specimen
- 8/9 Deliver six 20 μ l drops separately; five to one side and the last to the opposite side of a CLED plate (spread the latter)
- 10 Automatically pick up 500 μ l peptone
- 11 Manually pick up 500 μ l chloros
- 12 Manually deliver 1000 μ l of peptone/chloros