

ADHESION OF CELLS TO SURFACES COATED WITH POLYLYSINE

Applications to Electron Microscopy

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Many kinds of cells attach firmly and spread on solid surfaces to which polylysine has been adsorbed. The cells remain alive but may flatten or spread themselves to a degree that is not normal but may be desirable for observational purposes. Polylysine-coated plates or grids to which living cells are glued may conveniently be transferred through experimental solutions and through fixation and processing for scanning or transmission electron microscopy.

The principle behind the method is that the polycationic polylysine molecules adsorb strongly to various solid surfaces, leaving cationic sites which combine with the anionic sites on cell surfaces. In this laboratory, we derived the method from an earlier application: the use of protamine-coated surfaces to hold sea urchin eggs firmly for the insertion of microelectrodes (2). Other papers in the literature describe the behavior of cells on positively charged surfaces (e.g., reference 1). The present work has been reported briefly in an abstract, and here we supply the few details required for the practical use of the method.

Small squares of clean glass are used in preparations for scanning electron microscopy. Formvar-covered grids are used for work with the transmission electron microscope. Surfaces of plastics may be used; for example, cells adhere and spread well on polylysine-coated Falcon petri dishes (Falcon Plastics, Div., of B.-D. Laboratories, Los Angeles, Calif.).

The surface is prepared by covering it briefly with a 0.1% solution of poly-*l*-lysine in water. Our work has been done with polylysine of molecular weight 80,000–100,000 daltons obtained from New England Nuclear, Boston, Mass. A few trials sug-

gest that more dilute solutions of polylysine will serve as well.

The surface is thoroughly washed under running water. There is no danger of displacing the adsorbed layer of polylysine. The surface is then washed with a medium appropriate for the cells. The cell suspension is put on the surface and the cells attach as soon as they settle. Cells which have any tendency to spread do spread quite promptly. In some cases, the degree of spreading is greater than is seen on normal surfaces on which the cells grow. Such is the case with myoblasts and with amebas of *Dictyostelium*. Sea urchin eggs which have been prefixed in glutaraldehyde adhere to the polylysine surfaces. Other kinds of cells have not yet been tested.

The attached cells are not detached by treatment with the fixatives normally used for scanning and transmission electron microscopy or by the further processing, such as critical-point drying or negative staining. The application of the method to the processing of cells for light microscopy, e.g., squash or whole-mount Feulgen preparations, has been explored with some success. There is some risk of detachment of cells in the widely used alcohol-acetic (3:1) fixative.

One interesting application of the method is in the observation of the inner face of the cell surface. The cells are attached to the polylysine surface and subjected to shear in a medium in which the cytoplasm will disperse when the cell membrane is torn. The body of the cell is sheared away but the area of the cell surface which is glued by the polylysine remains attached, inner face up. Such a method has been employed by Vacquier (3) to observe the inner surfaces of sea urchin eggs after

the attached cells were torn by squirting a calcium-free medium at them. Fig. 1 shows an ameba of *Dictyostelium* prepared so that both the outside and inside surfaces can be observed in the scanning electron microscope.

Various experimental procedures can be carried out with cells thus glued to surfaces. An example is the treatment of attached flagella of sea urchin sperm with 1% Triton X-100 (Fig. 2). As the membrane disperses, the central microtubules and outer doublets attach to the surface and may be observed in detail with the transmission electron microscope.

SUMMARY

Cells of many kinds adhere firmly to glass or plastic surfaces which have been pretreated with polylysine. The attachment takes place as soon as



FIGURE 1 *Dictyostelium* amebas, $\times 3,500$. A suspension of amebas was permitted to spread on the polylysine surface and then squirted with a medium composed of 0.1 M KCl, 10 mM Tris, 1 mM $MgCl_2$, pH 7.5. Fixation was performed in this medium with 2.5% glutaraldehyde and 1% acrolein. The sample was postfixed in 1% OsO_4 in the medium, rapidly dehydrated in ethanol, transferred in graded series to Freon 113, and critical-point dried in Freon 13. The platinum-carbon-coated specimen was viewed in a field emission scanning electron microscope with an accelerating voltage of 15 kV. The uppermost cell is broken open to reveal the bottom of the cell's cortical layer. (Taken from the unpublished research of Margaret Clarke and Gerald Schatten.) Calibration bar = 1 μm .

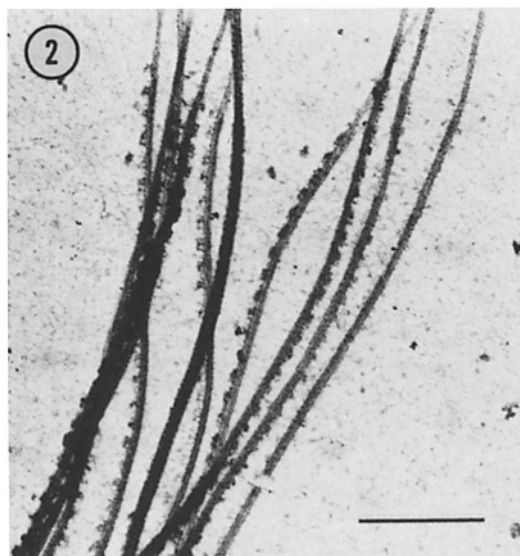


FIGURE 2 Spread ciliary axoneme, $\times 16,250$. Isolated *Tetrahymena* cilia were suspended in a medium containing 0.15 M KCl, 0.5 mM *B*-mercaptoethanol, 0.5 mM EDTA, 5 mM $MgSO_4$, and 2 mM Tris, pH 7.9, on a polylysine-coated grid. The grid was then immersed in 1% Triton X-100 for 15 s, then transferred to a fixative of 4% glutaraldehyde in Na cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 . The grid was then stained with 0.5% uranyl acetate in 50% ethanol for 20 min, dehydrated, and Freon 13 critical-point dried. This treatment will often result in spread ciliary axonemes in which the A and B subfibers, spokes, and central pair can be distinguished. Calibration bar = 1 μm .

the cells make contact with the surfaces, and the flattening of the cells against the surfaces is quite rapid. Cells which do not normally adhere to solid surfaces, such as sea urchin eggs, attach as well as cells which normally do so, such as amebas or mammalian cells in culture. The adhesion is interpreted simply as the interaction between the polyanionic cell surfaces and the polycationic layer of adsorbed polylysine. The attachment of cells to the polylysine-treated surfaces can be exploited for a variety of experimental manipulations. In the preparation of samples for scanning or transmission electron microscopy, the living material may first be attached to a polylysine-coated plate or grid, subjected to some experimental treatment (fertilization of an egg, for example), then transferred rapidly to fixative and further passed through processing for observation; each step involves only the transfer of the plate or grid from

one container to the next. The cells are not detached. The adhesion of the cell may be so firm that the body of the cell may be sheared away, leaving attached a patch of cell surface, face up, for observation of its inner aspect. For example, one may observe secretory vesicles on the inner face of the surface (3) or may study the association of filaments with the inner surface (Fig. 1). Subcellular structures may attach to the polylysine-coated surfaces. So far, we have found this to be the case for nuclei isolated from sea urchin embryos and for the microtubules of flagella, which are well displayed after the membrane has been disrupted by Triton X-100 (Fig. 2).

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